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NIH Research progress progress report

G. Bench

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RESEARCH PROGRESS

During the past year, the Resource continued to address three major gaps in the continued growth and application of AMS in biomedical research. The first identified gap is the lack of high throughput analysis coupled to molecular speciation, which can be solved by the development of an on-line fully integrated AMS-HPLC system. A second gap is the lack of robust methods for quantifying cellular metabolic endpoints for use in systems and cell biology applications. Understanding how to measure post-translational modifications, metabolic flux and metabolic rate with acceptable sensitivity, precision and reproducibility using substrate concentrations that are within the natural physiological range so that the normal metabolism is not perturbed is likewise important. This should become routine when the coupled AMS-HPLC system is available. Third, the ability to measure biological endpoints in humans is important and AMS offers the ability to do this safely. Our overall goal is to develop AMS and apply its quantitative capabilities to enable effective research in fundamental biology and human health. To that end, this cycle of Resource funding is devoted to pursuing the following specific aims:

1.) Increased throughput of AMS through direct coupling to separatory instruments.
    a.) Conversion of liquid HPLC eluents to gaseous CO_2, directly linking complex chemical analysis to the ion source.
    b.) Improve the capability to measure very small samples with high precision.

2.) Increase the value and information content of AMS measurements by combining molecular speciation with quantitation of defined isolates for pathway analysis from very small cellular, animal, and human samples.
    a.) Quantitate isotope content and flux in physical and chemical isolates of cells at the individual metabolite and at the pathway level.
    b.) Quantitate modified or derivatized macromolecules important in cellular metabolism, signaling and control.
    c.) Provide quantitation of biological systems using multiple isotopic tracers such as ^{3}H, ^{14}C and other isotopes measured at LLNL.

3.) Develop and validate methods for the use of AMS in translational human studies.
    a.) Demonstrate robust methods for conducting pharmacokinetic/pharmacodynamics in humans and model systems and coupling this information to computational modeling.

4.) Provide high throughput precision quantitation for collaborative and service clients.

To accomplish these aims we organized this center into 3 Core Research and Development Subprojects and a Collaborative and Service Center. Core Project 1 focuses on our aim to increase AMS throughput via development of a coupled AMS-HPLC system. Our hypothesis is that such a coupled system will significantly increase throughput and enable routine quantification of individual components of complex mixtures thus broadening AMS's utility for the biomedical sciences. Core Project 2 focuses on developing tools to quantitate important metabolic steps and subsystems in cells and to test the hypothesis that quantitative metabolic flux data will improve computational models of metabolic systems and their interactions. It will also work to quantify rates of protein metabolism and post-translational modifications of proteins in cells. Core Project 3 focuses on developing and validating methods to conduct studies in humans, mostly for use in drug development, nutrition and toxicology. This project is working to test the hypothesis that AMS data can improve the accuracy of physiologically-based pharmacokinetic models for predicting human effects of drugs/toxicants or nutrients and can enable earlier human testing of such compounds than is now possible. These core projects are linked to developing the technology to allow more rapid and cost effective analysis of metabolism in cells and higher organisms for use in understanding the dynamic processes that occur and how individual variation, disease, toxicants and drugs affect them. The collaborative and service center oversees the operation of our sample preparation process and accelerator mass spectrometry measurements for our collaborative and service users and assists with study design and data interpretation. These collaborative projects are coupled to the core research and development the center undertakes.

Resource Progress

This past year the three Technology Development projects of the Resource have continued to make good progress in pursuit of the Resource's specific aims. In addition, we have added new collaborative subprojects and they, along with our existing collaborators, are continuing to provide the scientific drivers for the Technology Development projects.

Updates of the Resource Technology Development projects are given below.

Project 1 (SPID 0001)

Project 1: Sample-Spectrometer Interface

PI: Ted Ognibene, Co-PIs: Bruce Buchholz, Ken Turteltaub, Kurt Haack

Project 1 seeks to improve our capability to quantitate very small samples. These capabilities will use interfaces developed for our new gas-accepting ion source, which was installed onto our existing 1-MV AMS system. This source makes more efficient use of the sample and requires less sample handling. It reduces the minimum sample size required for analysis while improving analysis throughput while lowering costs.

We are using the moving wire interface to convert small liquid samples to CO_2, which are injected into our gas-accepting ion source for ^{14}C-AMS analysis. We have made a number of measurements for a variety of collaborators. These measurements have been conducted as either discrete liquid drops or as directly-coupled HPLC-AMS analysis. Sample matrices have consisted of DNA, DNA adducts, cells, and metabolite fractions. We have developed stand-alone data analysis software to process results.

Quantitation of low-abundance protein modifications involves significant analytical challenges, especially in biologically-important applications; such as studying the role of post-translational modification in biology and measurement of the effects of reactive drug metabolites. ^{14}C labeling combined with accelerator mass spectrometry (AMS) provides exquisite sensitivity for such experiments. Recently, we demonstrated real-time ^{14}C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS). By enabling direct HPLC-AMS coupling, LS-AMS overcomes several major limitations of conventional HPLC-AMS; where individual HPLC fractions must be collected and converted to graphite before measurement. To demonstrate LS-AMS and compare the new technology to traditional solid sample AMS (SS-AMS), reduced and native bovine serum albumin (BSA) was modified by ^{14}C-iodoacetamide, with and without glutathione present, producing adducts on the order of 1 modification in every 10^6 to 10^8 proteins. ^{14}C incorporated into modified BSA was measured by solid carbon AMS and LS-AMS. Reduced and native BSA peptides were generated by tryptic digestion. Analysis of HPLC-separated peptides was performed in parallel by LS-AMS, fraction collection combined

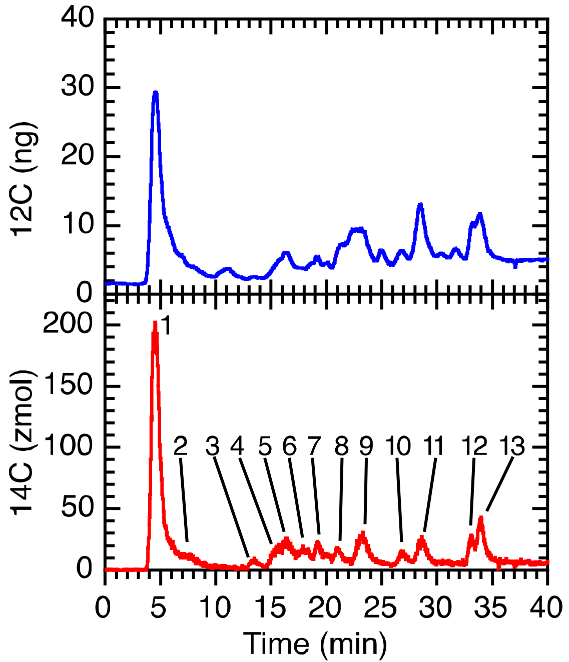

Figure 1. LS-AMS ^{12}C (top) and ^{14}C (bottom) traces of peptides from ^{14}C-iodoacetamide-modified reduced BSA. Numbered peaks correspond to cysteine-containing peptides.

with SS-AMS, and (for peptide identification) electrospray ionization and tandem mass spectrometry (ESI-MS/MS). LS-AMS enabled ^{14}C quantitation from ng sample sizes and was 100 times more sensitive to ^{14}C incorporated in HPLC-separated peptides than SS-AMS, resulting in a lower limit of quantitation of 50 zmol ^{14}C/peak. Additionally, LS-AMS turnaround times were minutes instead of days, and HPLC trace analyses required 1/6th the AMS instrument time required for analysis of graphite fractions by SS-AMS.

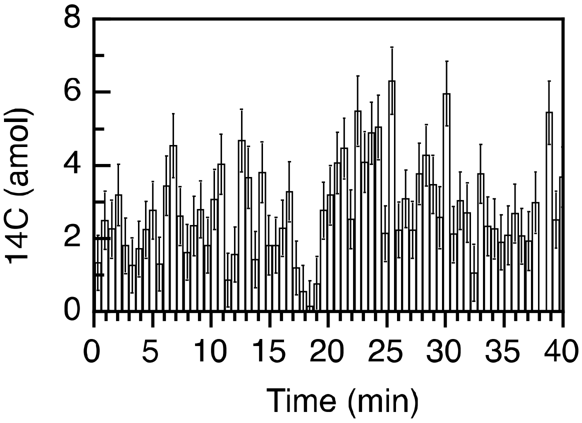

Figure 2. SS-AMS spectrum from an HPLC separation of peptides from ^{14}C-iodoacetamide-modified reduced BSA. Each column represents one 45-sec fraction, which was collected and converted to graphite after the addition of carbon carrier.

Figure 1 shows a representative spectra of ^{12}C (top) and ^{14}C (bottom) traces of LS-AMS measurements of HPLC-separated peptides from reduced BSA modified by ^{14}C-iodoacetamide. The following numbered ^{14}C peaks were found by MS/MS to correspond to the following cysteine-containing peptides: 2, ETYGDMADCCEK; 3, TCVADESHAGCEK and QNCDQFEK; 4, ETYGDMADCCEK and SHCIAEVEK; 7, LKPDPNTLCDEFK and YNGVFQECCQAEDK; 8, RPCFSALTPDETYVPK and SLHTLFGDELCK; 10, GLVLIAFSQYLQQCPFDEHVK and LFTFHADICTLPDTEK. All other numbered peaks have elution times corresponding to more than two Cys-containing peptides.Conversely, no substantially elevated fractions, above the carbon carrier background, were detected by solid carbon AMS from the ^{14}C-iodoacetamide-reduced separation as presented in Figure 2. Failure to detect elevated fractions from the ^{14}C-IAC-reduced separation is due to a lack of sensitivity, arising from the combination of extremely low-level labeling of numerous peptides and the presence of ^{14}C from the tributyrin carbon carrier present in all fractions.

For LS-AMS analysis, effluent from the HPLC was applied directly to the interface. For solid carbon AMS, as the HPLC separations contained only ~15 μg C, it was necessary to add 1 μL of tributyrin carrier, containing 0.61 mg C with an isotopic ratio of 11.2 ± 0.3 amol ^{14}C/mg ^{12}C (1 amol = 10^{-18} mol), to each fraction, to provide sufficient carbon for graphitization and AMS analysis. Under this regime, the amount of carbon from the carrier dominates the ^{12}C from the sample and 1 μL of tributyrin carrier adds 6.8 ± 0.4 amol ^{14}C to each fraction. ^{14}C from the HPLC eluate in each fraction was determined by subtracting the measured isotopic ratio of the carrier from the measured isotope ratio of each fraction, then multiplying by the carbon mass of the carrier. This process adds a significant uncertainty to the ^{14}C measurement. As a result, the uncertainty of individual solid carbon AMS fraction measurements is approximately ±1 amol ^{14}C/fraction while the lower limit of quantitation (LLOQ) is 10 amol ^{14}C/fraction. In contrast, LC-LS-AMS ^{12}C backgrounds were between 2 to 4 ng/second and ^{14}C background was less than 10^{-3} amol ^{14}C/second with a LLOQ of 50 zmol ^{14}C/peak (1 zmol = 10^{-21} mol ≈600 atoms).

LC-LS-AMS also provided superior temporal resolution compared to fraction collection combined with solid carbon AMS. For LC-LS-AMS, the integrated ^{12}C charge and ^{14}C counts are recorded at 1 second intervals. Peak maximum locations could be determined to the nearest second and varied by less than 5 seconds between replicates. Typical peak widths, measured by full width at half maximum, were in the range of 35 seconds and varied by less than 2 seconds between replicates. Without employing complex peak fitting algorithms, the temporal resolution of solid carbon AMS analysis is limited by the fraction collection time. Increasing the number of fractions can improve temporal resolution, but this would require more carrier carbon and thus decrease sensitivity and decrease analysis throughput.

In addition to improved sensitivity, improved temporal resolution and reduced sample preparation, traces measured by LC-LS-AMS took significantly less AMS instrument time to measure: ~1 hour per trace. In contrast, the low ^{14}C content and dilution by carrier carbon of the solid carbon fractions (resulting in an average isotopic ratio of ~15 amol ^{14}C/mg C) required ~6 hours of AMS instrument time per separation to measure the 75 fractions collected from the separation shown in Figure 2.

*Thomas, A. T., B. J. Stewart, T. J. Ognibene, K. W. Turteltaub and G. Bench (2013). "Directly Coupled High-Performance Liquid Chromatography-Accelerator Mass Spectrometry Measurement of Chemically Modified Protein and Peptides." Analytical Chemistry **85**(7): 3644-3650.

Project 2 (SPID 0002)

Project 2: Quantitative Cell Biology

PI: Graham Bench, Co-PIs: Ben Stewart, Bruce Buchholz

Significant effort in biology is devoted towards understanding how molecules are metabolized and interact at the covalent and non-covalent levels. These interactions are involved in cell-cell communication, cell regulation and toxicity, among others, and thus impact many areas of biomedical interest. Numerous studies are directed at identifying molecules that play a role in these processes and toward gaining insight into how each process impacts function. In many cases it has been difficult to carry out such studies due to a lack of adequate tissue or cell sample size for analysis, effector concentrations that are too low to measure, and problems in tracing specific reactions in the complex mixture of molecules in cells even when high doses of label have been used.

AMS can play a unique role where cellular receptors or other effector molecules of interest may be expressed in low numbers or otherwise present at low concentrations, and can play a role in characterizing metabolic or other reaction mechanisms when the products are unknown. Successful use of AMS not only requires adequate sample preparation methods, but also requires that the biological aspects of the experiments be designed and conducted for compatibility with AMS analysis. In the previous funding cycle, Core Project 2: Cell Biology, focused on gaining an understanding of both the composition and function of individual cells, how cells change over their lifetime and in response to signals from their environment as well as determining how cells form a whole organism. We proposed to develop methods to couple cell biology experiments to AMS and to quantitate components speciated from pools of cells labeled with ^{14}C. Four specific aims were proposed:

1. Isolate sub-cellular fractions and speciate metabolites from pools of cells.
2. Couple flux balance analysis to AMS using yeast as a model organism.
3. Couple AMS to quantitative measurement of post-translational modifications.
4. Couple AMS to cell turnover in human tissues using the ^{14}C bomb curve.

C1.2.1) Isolate sub-cellular fractions and speciate metabolites from pools of cells and C1.2.2) Couple flux balance analysis to AMS using yeast as a model organism.

Research performed under Specific Aims 1 and 2 made use of the yeast Saccharomyces cerevisiae as a model system for development of metabolite separation and speciation techniques and the demonstration of AMS for measurement of intracellular amino acid fluxes. Yeast labeling protocols and metabolite extraction and separation techniques were developed to measure intracellular and extracellular amino acids. External and internal metabolic fluxes culminating in the production of the antioxidant glutathione were measured in yeast using accelerator mass spectrometry and HPLC. These experimental results were then used to significantly improve a mathematical model of yeast metabolism. Internal fluxes were shown to be substantially more information-rich than external fluxes (Stewart, et al., 2010). Additional experiments were performed to investigate metabolism of methylglyoxal, a toxic dicarbonyl generated by the spontaneous degradation of glycolytic intermediates. We performed experiments using the model organism *Saccharomyces cerevisiae* grown in media containing low, moderate, and high glucose concentrations to determine the relationship between glucose consumption and methylglyoxal metabolism. Normal growth experiments and glutathione depletion experiments showed that metabolism of methylglyoxal by log-phase yeast cultured aerobically occurred primarily through the glyoxalase pathway. Growth in high-glucose media resulted in increased generation of the methylglyoxal metabolite D-lactate and overall lower efficiency of glucose utilization as measured by growth rates (Stewart, et al., 2013).

C1.2.3) Couple AMS to quantitative measurement of posttranslational modifications.

Quantitation of low-abundance protein modifications involves significant analytical challenges, especially in biologically important applications such as studying the role of post-translational modification in biology and measurement of the effects of reactive drug metabolites. Research performed under Specific Aim 3 involved measurement of proteins and peptides alkylated with ^{14}C-iodoacetamide and measurement by liquid chromatography-tandem mass spectrometry and liquid sample AMS. Labeling techniques and separation methods were developed to allow measurement of proteins and peptides using the liquid sample interface. We performed real-time ^{14}C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LSAMS). We demonstrated that direct HPLC-AMS coupling overcomes several major limitations of conventional HPLC-AMS where individual HPLC fractions must be collected and converted to graphite before measurement. To demonstrate LS-AMS and compare the new technology to traditional solid sample AMS (SS-AMS), reduced and native bovine serum albumin (BSA) was modified by 14C-iodoacetamide, producing adducts on the order of 1 modification in in every 106 to 108 proteins. ^{14}C incorporated into modified BSA was measured by solid carbon AMS and LS-AMS. Reduced and native BSA peptides were generated by tryptic digestion with analysis of HPLC-separated peptides performed in parallel by LS-AMS, fraction collection combined with SS-AMS, and (for peptide identification) electrospray ionization and tandem mass spectrometry (ESI-MS/MS). These results were published in the journal Analytical Chemistry (Thomas, et al., 2013).

C1.2.4) Couple AMS to cell turnover in human tissues using the ^{14}C bomb curve. Projects coupling AMS to cell and protein turnover in human tissues using the ^{14}C bomb curve were advanced in Specific Aim 4. Interest in this technique continues to grow as evidenced by the growing number of collaborators wishing to explore this technique. Specific sample definition is the biggest challenge in these dating and turnover projects. Four general classes of samples were studied: DNA, proteins, lipids, and dental enamel. We developed a method for the retrospective birth dating of cells using bomb pulse ^{14}C dating as a method for measuring the approximate age of specific populations of cells in the adult human brain and other tissues (Spalding, et al., 2005). This method is based on establishing the proportion of the isotope ^{14}C in genomic DNA. After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA reflects the age when the cell was born. Atmospheric nuclear tests in the late 1950s and early 1960s doubled the level of ^{14}C in the atmosphere followed by an exponential decline since 1963, allowing one to resolve ^{14}C differences in the range of years. Because DNA has a ^{14}C content reflective of the time when it was synthesized, establishing the ^{14}C content of chromosomal DNA enables retrospectively birth dating of cells, and thus establishes cellular turnover. Establishing a marker to specifically identify and separate specific cell types has been the biggest challenge in these studies. Pancreatic beta cells were separated using pro-insulin as a marker to establish turnover ceases after age 30 (Perl, et al., 2010). Long time collaborator at the Karolinska Institute, Kirsty Spalding, continues to probe specific brain regions for neurogenesis using NeuN+ as a positive nuclear surface marker for neurons. Spalding has also advanced investigations of lipid turnover in health and disease to determine that lipid turns over every 1.5 years (Arner, et al., 2011) while fat cells live about 10 years (Spalding, et al., 2008) in healthy individuals. The carbon in most proteins is replaced relatively rapidly. The lens of the eye is an exception, with cells and structural proteins formed *in utero* lasting a lifetime. The lens grows throughout life adding cells to the outer layers. Using relatively simple separation of water-soluble (crystalline) proteins from water-insoluble (mostly membrane) and molecular weight spin filters to remove small molecules, Stewart DN, et al. (2013) quantified turnover of water-soluble crystalline proteins to be ~0.5-1% annually while membrane proteins were not renewed. The finding is significant because it contradicts dogma and demonstrates a natural process of renewing carbon in elderly crystalline proteins. We also recently developed a technique to extract collagen from blood vessels. Collagen is typically extracted from bone by dissolving the mineral phase in acid and used to date archeological remains. Here collagen was extracted from blood vessels to date ruptured and unruptured intercranial aneurysms (Etminan, et al., 2013). The collagen was less than 5 years old in all cases with larger aneurysms being slightly older. Finally, bomb pulse dental enamel to determine date of birth in forensic cases has been systematically extended by combining enamel dating with other analyses. Aspartic acid racemization of teeth can provide an estimate of date of death when combined with enamel dating (Alkass, et al., 2010). Stable isotope analysis can provide geographical information of residence during tooth formation (Alkass, et al., 2011; Alkass et al., 2013). As the forensic community becomes aware of enamel dating, it is being applied to cold cases with other modern analytical techniques to solve cases (Speller, et al., 2012). During the next proposed funding cycle we expect more effort to explore molecule-specific age dating using the new interface and exploring techniques on new tissues.

Meetings Attended: Society of Toxicology Annual Meeting

Poster Presentation: Absolute Quantitation of Low-Abundance Protein Adducts using a Novel Accelerator Mass Spectrometry Liquid Sample Interface, Society of Toxicology meeting, 12 March 2013.

Publications:

Thomas AT, Stewart BJ, Ognibene T, Turteltaub KW, Bench G: **Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides**. *Anal Chem* 2013, **85**(7):3644-3650.

Stewart BJ, Navid A, Kulp KS, Knaack JL, Bench G: **D-Lactate production as a function of glucose metabolism in Saccharomyces cerevisiae**. *Yeast* 2013, **30**(2):81-91.

N. Etminan, B.A. Buchholz, R. Dreier, P. Bruckner, J.C. Torner, H.-J. Steiger, D. Hänggi, R.L. Macdonald. **Cerebral aneurysms: Formation, progression and developmental chronology**. *Translational Stroke Research* 2014, Published online 30 October 2013. 10.1007/s12975-013-0294-x

K. Alkass, H. Saitoh, B.A. Buchholz, S. Bernard, G. Holmlund, D.R. Senn, K.L. Spalding, H. Druid. **Analysis of radiocarbon, stable isotopes and DNA in teeth to facilitate identification of unknown decedents**. *PLoS ONE* 2013, **8**:e69597. DOI: 10.1371/journal.pone.0069597.

K.L. Spalding, O. Bergmann, K. Alkass, S. Bernard, M. Salehpour, H. Huttner, E. Boström, I. Westerlund, B.A. Buchholz, G. Possnert, D. Mash, H. Druid, J. Frisén. **Dynamics of hippocampal neurogenesis in adult humans**. *Cell* 2013, **153**:1219-1227.

D.N. Stewart, J. Lango, K.P. Nambiar, M.J.S. Falso, P.G. FitzGerald, B.D. Hammock, B.A. Buchholz. **Carbon turnover in water-soluble protein of the adult human lens**. *Molecular Vision* 2013, **19**:463-475.

N. Etminan, R. Dreier, B.A. Buchholz, P. Bruckner, H.-J. Steiger, D. Hänggi, R.L. MacDonald. **Exploring the age of intercranial aneurysms using carbon birth dating: preliminary results**. *Stroke* 2013, **44**: 799-802.

Project 3 (SPID 0003)

Project 3: Quantitative Endpoints in Humans

PI: Mike Malfatti (LLNL), Co-PIs: Paul Henderson (UC Davis), Ken Turteltaub (LLNL)

This project focuses on developing and validating methods and techniques to demonstrate the applicability of AMS to assess human responses to drugs, xenobiotics and endogenous compounds. We are conducting studies using AMS to develop and validate methods for use in absorption, distribution, metabolism and elimination studies (ADME) using drugs alone and in combination. We will investigate how best to conduct basic metabolism studies as well as to work with isolated macromolecular fractions such as DNA modified by chemotherapy drugs. We will work in cell culture, animal models, and humans to assess what types of samples can be utilized and how best to process them for AMS analysis.

Nanoparticles (NP) and their use as diagnostic and therapeutic agents in the biomedical field are becoming increasingly more popular. However, to date, the toxicity and biological fate have not been thoroughly investigated. Iron oxide nanoparticles are utilized for many bio-applications, which include: imaging, as drug delivery vehicles and for cell tracking. In this work, Accelerator Mass Spectrometry (AMS), an ultrasensitive technique for quantifying rare isotopes, is used to quantify the biodistribution and pharmacokinetic properties of ^{14}C-labeled iron oxide NP *in vivo*.

^{14}C-labeled carboxylated iron oxide NP (~10nm core size) were administered by nose only inhalation (0.175mg) or by a bolus intravenous dose (IV) (0.15mg) to male mice. For inhalation delivery, over 7 d, NP were observed to clear primarily from the lungs through the gastrointestinal system for excretion into the feces ($t_{1/2}$ = 1.42 d). Detectable levels were also observed in plasma and phagocytic organs such as the liver and spleen. Furthermore, accumulation in the olfactory bulb was also observed (1.05 ng/mg at 7d). After intravenous delivery, NP were observed to accumulate primarily in the liver, spleen and lungs over a 24 h period; the half-life in plasma was $t_{1/2}$ = 6.4 h. Taken together, these data indicate that once administered, carboxylated iron oxide NP are absorbed and distributed to major organs and are retained in tissue through 24 h for IV and through 7 d for inhalation. These observations may provide insight for assessment of potential toxicity upon exposure to iron oxide nanoparticles**.**

Platinum microdosing

To date sixteen bladder and lung cancer patients have been given microdoses of [14C]carboplatin 24h prior to a biopsy. Select patients also gave blood samples over 24 h in order to measure the plasma pharmacokinetics of the microdose. Three patients were dosed twice, once each with a [14C]microdose and once each with a [14C]therapeutic dose. The pharmacokinetics of all patients were nearly identical (within 2-fold regarding t1/2 alpha and t1/2beta). The PK were also essentially identical between microdose and therapeutic dose experiments. So far, 10 of the patents went on to full dose platinum-based chemotherapy, and we have response data for six patients. The lowest drug-DNA adduct levels were from patients the had progression (no response to chemotherapy), whereas higher drug-DNA adduct levels correlated with mixed responses (ranging from one non responder, one with residual cancer and two complete responses). We are now expanding the study to hospitals at UCLA, USC and UCSF in order to increase accrual to the point where the correlations become statistically significant.

We also started an oxaliplatin/breast cancer microdosing project, which just opened to accrual at UC Davis on March 3, 2014.

RESEARCH HIGHLIGHTS

The Resource has chosen to highlight progress and publications from one core project and two collaborative projects for this year's progress report.

Highlight #1:

SPID: 0025

Subproject Title: Neuronal Turnover in the adult brain using a novel method for retrospective birth dating of cells

Investigators: Jonas Frisen, Bruce Buchholz

Using data derived from nuclear weapons testing of the 1950s and '60s, Lawrence Livermore scientists have found that a small portion of the human brained involved in memory makes new neurons well into adulthood.

The research may have profound impacts on human behavior and mental health. The study supports the importance of investigating the therapeutic potential of applying adult neurogenesis to the treatment of age-related cognitive disorders.

Neurogenesis is the process by which neurons are generated from neural stem and progenitor cells and are believed to be most active during pre-natal development.

Until now. LLNL's Bruce Buchholz, colleagues from the Karolinska Institute and international collaborators found that hippocampal neurogenesis occurs at significant levels through adulthood and into old age. (Humans and other mammals have two hippocampi, one in each side of the brain.)

The hippocampal portion of the brain is thought to affect memory. Research has shown that people with a damaged hippocampus can have a variety of memory issues including long-term memory loss, loss of conversion of short-term to long-term memory or no spatial navigation.

The team used radiocarbon dating techniques, typically used in archaeology, to date cells in the hippocampus. The technique is based on the spike in global levels of carbon-14 (14C) that resulted from extensive above-ground nuclear weapons testing during the Cold War. Since the test ban, there has been a steady decline in atmospheric 14C.

Because plants absorb 14C via CO2 during photosynthesis, animals that eat them also take in radioactive carbon; therefore, the 14C level in the human body reflects that of the atmosphere. When a cell divides, newly synthesized DNA integrates a trace amount of 14C that is proportional to the environmental level at the time of mitosis; so, the radioactivity of a cell nucleus can be used as a time stamp of the cell's genesis.

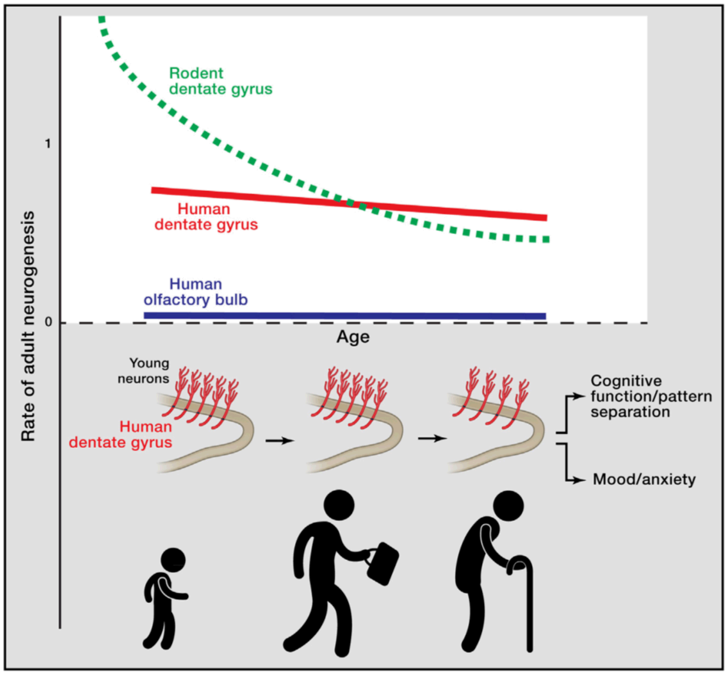

Figure 1. Comparative Rates of Adult Neurogenesis in Mice and Men
In contrast to the very low levels of adult neurogenesis reported in the human olfactory bulb (blue line), the human hippocampus continues to generate neurons at a steady rate well into old age with only a modest decline throughout adulthood (red line). The rate of neurogenesis in adult humans is comparable to levels seen in middle-aged rodents (9 months old; intersection of green line and red line). These results suggest that studies in rodents revealing the role of adult hippocampal neurogenesis in cognitive function (pattern separation) and emotional behavior (mood/anxiety) may also hold true in adult humans.

Using middle-aged mice for the experiment, the team found specifically that one third of hippocampal neurons are subject to exchange with an annual turnover rate of 1.75 percent (1,400 new neurons are added every day) in adults with a modest decline in older individuals. The team then tested tissue from postmortem hippocampi of human subjects.

"We conclude that neurons are generated throughout adulthood and that the rates are comparable in middle-aged humans and mice, suggesting that adult hippocampal neurogenesis may contribute to human brain function," said LLNL's Bruce Buchholz who performed the radio carbon dating for the study.

Researchers said the individuals showed significant variability in levels of incorporated 14C, which could serve as an opportunity to retrospectively compare levels of hippocampal neurogenesis with each individual's medical history in order to probe for a relationship between psychiatric conditions and rates of cell turnover.

Other institutions include: University of Lyon, Uppsala University, University of Erlangen-Nuremberg and University of Miami.

The research appears in the June 6 edition of the journal, *Cell*.

The study was supported by the Swedish Research Council, T. Stiftelsen, Hjarnfonden, the Swedish Foundation for Strategic Research, the National Alliance for Research on Foundation, the National Institute on Drug Abuse, the European Research Council, the National Institutes of Health (NIH) and the National Center for Research Resources.

*Excerpt above taken from LLNL Newsonline; Figure and caption taken from Kheirbeck and Hen, *Cell* Preview, June 2013

Highlight #2:

SPID: 0001

Subproject Title: Sample-Spectrometer Interfaces

Investigators: PI: Ted Ognibene, Co-PIs: Bruce Buchholz, Ken Turteltaub, Kurt Haack

Quantitation of low-abundance protein modifications involves significant analytical challenges, especially in biologically-important applications; such as studying the role of post-translational modification in biology and measurement of the effects of reactive drug metabolites. ^{14}C labeling combined with accelerator mass spectrometry (AMS) provides exquisite sensitivity for such experiments. Here we demonstrate real-time ^{14}C quantitation of HPLC separations by liquid sample accelerator mass spectrometry (LS-AMS). By enabling direct HPLC-AMS coupling, LS-AMS overcomes several major limitations of conventional HPLC-AMS; where individual HPLC fractions must be collected and converted to graphite before measurement. To demonstrate LS-AMS and compare the new technology to traditional solid sample AMS (SS-AMS), reduced and native bovine serum albumin (BSA) was modified by ^{14}C-iodoacetamide, with and without glutathione present, producing adducts on the order of 1 modification in every 10^6 to 10^8 proteins. ^{14}C incorporated into modified BSA was measured by solid carbon AMS and LS-AMS. Reduced and native BSA peptides were generated by tryptic digestion. Analysis of HPLC-separated peptides was performed in parallel by LS-AMS, fraction collection combined with SS-AMS, and (for peptide identification) electrospray ionization and tandem mass spectrometry (ESI-MS/MS). LS-AMS enabled ^{14}C quantitation from ng sample sizes and was 100 times more

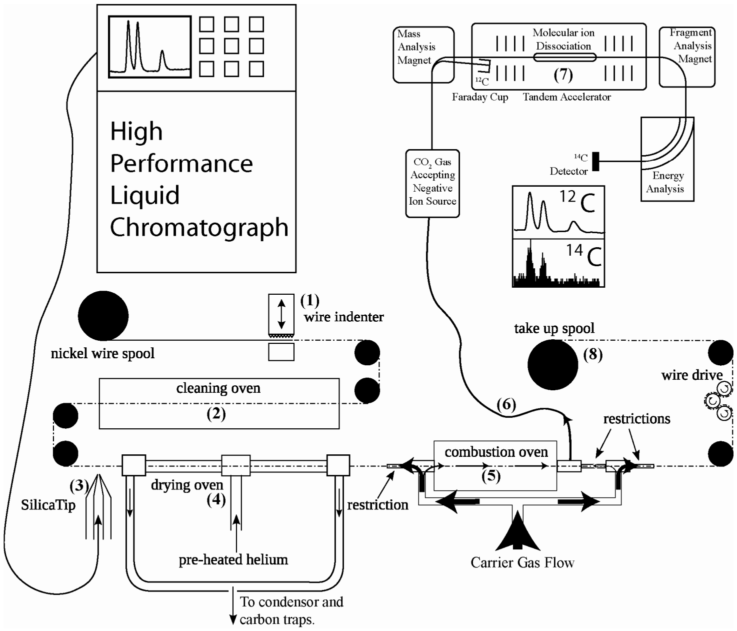
High Performance Liquid Chromatograph
Mass Analysis Magnet
Molecular ion Dissociation
Fragment Analysis Magnet
^{12}C
(7)
Faraday Cup
Tandem Accelerator
CO$_2$ Gas Accepting Negative Ion Source
^{14}C Detector
Energy Analysis
^{12}C
^{14}C
(1)
wire indenter
nickel wire spool
cleaning oven
(2)
take up spool
(8)
wire drive
(6)
restrictions
combustion oven
(5)
(3)
SilicaTip
drying oven
(4)
restriction
pre-heated helium
Carrier Gas Flow
To condensor and carbon traps.

Figure 2. Schematic layout of LS-AMS. (1) Wire indenter generates periodic indentations on the wire. (2) Surface carbon is removed and the wire is oxidized at high temperature. (3) A stream of effluent or single droplet is placed on the wire. (4) Solvent is evaporated at elevated temperature in an atmosphere of helium. (5) Non-volatile analyte is combusted at high temperature in a helium and oxygen atmosphere. (6) The flow of gas through the combustion oven directs all combustion products to the gas accepting ion source through a narrow i.d. fused silica capillary. (7) The ^{14}C and ^{12}C content of the CO$_2$ is measured using a gas accepting cesium sputter ion source followed by AMS analysis. (8) Used wire is collected on a separate spool for disposal, preventing cross contamination.

sensitive to ^{14}C incorporated in HPLC-separated peptides than SS-AMS, resulting in a lower limit of quantitation of 50 zmol ^{14}C/peak. Additionally, LS-AMS turnaround times were minutes instead of days, and HPLC trace analyses required 1/6th the AMS instrument time required for analysis of graphite fractions by SS-AMS.

Thomas, A. T., B. J. Stewart, T. J. Ognibene, K. W. Turteltaub and G. Bench (2013). "Directly Coupled High-Performance Liquid Chromatography-Accelerator Mass Spectrometry Measurement of Chemically Modified Protein and Peptides." Analytical Chemistry **85(7): 3644-3650.*

Highlight #3:

SPID: 00068

Subproject Title: Transport of ^{14}C-TCC across the placental barrier

Investigators: Heather Enright, Bruce Buchholz, Mike Malfatti,

It has been demonstrated that a variety of chemicals are not removed after wastewater treatment, which results in their release back into the water supply and environment. These chemicals may act as endocrine disrupting compounds (EDC), which can affect the function of the endocrine system and adversely affect progeny. To date, studies evaluating the effects of EDC during periods of development are lacking, including quantitative measures of accumulation after exposure. In this work, we are quantifying the transfer and accumulation of the EDC, triclocarban (TCC), from mother to offspring both *in utero* and through lactation using accelerator mass spectrometry (AMS). The high sensitivity of AMS allows for assessment of environmentally relevant concentrations of TCC (pM-nM).

^{14}C-labeled TCC was administered to pregnant female mice through their drinking water (100nM) during gestation or lactation using custom made water bottles. As shown in Table 1, detectable quantities of TCC were found in offspring for both exposure groups with the lactation group having three times the concentration (PND10 = 0.015%ID/g) than the gestation group (GD18 = ~0.005%ID/g). An increase in average offspring weight for both groups was also noted when compared to their control counterparts (Table 2); this increase was more significant for the lactation group, which contained the highest concentrations of TCC.

Taken together, these data indicate that TCC is transferred from mother to offspring through two routes: across the placental barrier and through lactation and that this exposure is associated with increases in offspring weight. While these effects were observed in mice, these findings suggest that if TCC is similarly transferred in humans, there may be implications after exposure on human health, in particular obesity. Currently, we are investigating whether this observed increase in weight persists through adulthood and determining the bio-distribution of TCC within these offspring.

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Tissue	Average %ID/g tissue	SEM
Fetal Placenta	0.011	0.001
Maternal Placenta	0.007	0.001
Fetus GD18	0.005	0.001
PND 10	0.015**	0.002

Table 1. Average concentrations of TCC in tissue for *in utero* (n=6) and lactation (n=8) exposure (%ID = ingested dose). For *in utero* exposure, fetal and placental tissues were analyzed at gestational day 18 (GD18). Neonatal mice were exposed to TCC through lactation through post-natal day 10 (PND 10). **p<0.01

Group	Average weight (g)	SEM
TCC-*in utero*	1.26*	0.03
Ctrl-*in utero*	1.17	0.04
TCC-lactation	5.72***	0.10
Ctrl-lactation	4.30	0.17

Table 2. Average weight of offspring for exposure to TCC *in utero* (n=92) or during lactation (n=103). *p<0.05, ***p<0.001

ADVISORY COMMITTEE

External National Advisory Committee

The External National Advisory Committee is in place to oversee the Resource's scientific direction and operating policies. In addition, it comments on the needs and plans for adequate instrumentation, technology development, and future scientific direction. It helps recruit investigators for collaboration in new areas of research. The makeup of the committee was weighted by expertise in mass spectrometry to help guide the needs in technology development. It was also weighted by expertise in carcinogenesis and pharmacology/toxicology since these had been the theme areas for the Resource during its initial funding period. The membership of the External National Advisory Committee is currently being reviewed to ensure that the external expertise reflects the current scientific focus areas. A.L Burlingame, Ph.D., a senior investigator at University of California at San Francisco and expert in biomedical applications of mass spectrometry, has continued to serve as Chairman of the Committee. The members of the committee are listed in Table 1 below.

Table 1. Resource National Advisory Board Membership and Expertise

Committee Member	***Institution***	***Expertise***
B. Hammock	University of California, Davis	Cancer Etiology/ Pharmacology/ Toxicology
T. A. Baillie	University of Washington	Mass Spectrometry /Drug Metabolism
A. L. Burlingame	University of California, San Francisco	Biological Mass Spectrometry
J. T. Brenna	Cornell University	Nutrition/ Mass Spec Interfaces
James Felton	University of California, Davis	Toxicology
J. Davis	LLNL/ Hertz Foundation	Accelerator Mass Spectrometry
I. Cristea	Princeton University	Molecular Biology Proteomics

Internal Executive Committee

The Internal Executive Committee members are from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Lawrence Livermore National Laboratory (LLNL) Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues relating to Biomedical AMS. The members of the committee are listed below.

Member's Name	**Department**
Kenneth W. Turteltaub, Ph.D. Principal Investigator	Biosciences & Biotechnology Division
Graham Bench, Ph.D. Co- Principal Investigator	Center for Accelerator Mass Spectrometry
Bruce A. Buchholz, Ph.D.	Center for Accelerator Mass Spectrometry
Mike Malfatti, Ph.D.	Biosciences & Biotechnology Division
Ted J. Ognibene, Ph.D.	Center for Accelerator Mass Spectrometry
Kristen S. Kulp, Ph.D.	Biosciences & Biotechnology Division
Gabriela Loots, Ph.D.	Biosciences & Biotechnology Division

The External Advisory Committee, in conjunction with the Internal Executive Committee, strives to meet once a year. Additionally, Partial Committee meetings can occur on *ad hoc* occasions, such as workshops. Discussions also occur as needed by phone, email, or fax. The Annual Advisory Committee Meeting focuses on the progress being made on the Technology Development projects, the allocation of resources, and the Resource’s future.

Advisory Committee Meeting

This year’s advisory committee meeting was held on November 12, 2013. Five of the committee members were in attendance: Tom Baillie, University of Washington; Tom Brenna, Cornell University; Al Burlingame, UCSF; Jim Felton, LLNL retiree; and our newest member, Ileana Cristea from Princeton. The focus of the meeting was preparation for the upcoming site visit for the grant renewal. The agenda for the day and comments follow. There was no formal committee report generated by the committee.

Research resource for Biomedical Mass Spectrometry
Advisory Visit
November 12 2013

7.30- 8:00	Continental Breakfast for reviewers	
8:00- 8:15	Welcome and Introductions	Ken Turteltaub
8:15 – 9:00	Theme and Overview	Ken Turteltaub
9:00 – 9:45	TR&D 1	Ben Stewart
9:45 – 10:30	TR&D 2	Mike Malfatti
10:30- 10:45	Break	
10:45 – 11:30	TR&D 3	Ted Ognibene
11:30 – 1:00	Lunch and Executive Session	
1:00 – 1:30	DBP7 Tumor Metastatic Potential	Gaby Loots
1:30 -2:00	DBP6 Predicting Chemotherapy Efficacy	Paul Henderson
2:00- 2:30	Laser Based 14C	Daniel McCartt
2:30 – 3:00	Administration, Training and Dissemination	Kris Kulp
3:00- 3:15	Break	
3:15 – 3:30	Collaborative and service	Bruce Buchholz
3:30 – 3:45	Summary	Ken Turteltaub
3:45 – 4:45	Executive Session	
4:45- 5:15	Call back	
5:15	Dinner	

Notes from Advisory Board Visit
November 12, 2013

Introduction

- Take credit for accomplishments and potential for moving wire
- Written proposal suggests no cost reduction to moving wire because of increased sample number. Can we estimate cost/ HPLC trace to compare to graphite?
- Routine methods development vs "exotic methods" development- where do we put our time?
- Emphasize capabilities for routine analysis: consider tighter standards for brining in routine samples for analysis (electrospray as a model) – putting sample cleanup on the user with clear standards could help AMS penetrate the biomedical field more effectively
- Set up a pipeline that always works well and just run samples
- Emphasize that routine work flows exist (not re-inventing the wheel)
- Emphasize using the platform to penetrate areas of biology not previously accessible
- Emphasize the science in the DBPs; these are fundamentally important projects that are answering important questions that can only be answered this way
- Explain how they were chosen; not just "pulling tickets"; what characteristics drove the choices?
- Take ownership of the science resulting from the DBPs and put them in context of the even bigger bio picture (explain why they are important and why they were chosen)
- Take credit for the CAMS' huge impact on pharma through Acceleron and Vitalia
- FIX: change Kristy to Kirsten Spalding

TR&D1:

Aim1:

- Need appropriate timing for explaining how bomb pulse works
- Liposuction tissue; how will you handle it and deal with it? What do you do to deal with the complexity of liposuction tissue;
- Possibly mention techniques in lipidomics that can be used for moving wire
- Be careful if using lipids for validation; they are sticky and the volatility may be of issue
- Total triglycerides may be better than analyzing many peaks
- More clear on work flow and results
- What will you measure that is informative and important? Representative classes. (make clear it is class separation, not individual types)

Aim 2:

- Put in perspective what AMS contributes beyond what the commonly used method do
- What is this adding to the field?
- Emphasize that solving this problem can be used as a basis for solving other problems, bigger than histones
- Make clear it will be developed in yeast, but quickly move to more relevant cell types

Aim 3:
- Photoaffinity label- lots of discussion about specificity of label –
- Make clear what you want to learn from this
- Driver is fundamental bio question to identify receptor, but is the approach the best?
- What is the advantage of AMS compared to more traditional approaches? (e.g., how does this compare with SRM approach? 100x more sensitive, etc.)

Aim 4:
- What will be stoichiometry of labeled protein?
- Written proposal not clear how KumaMax is working in Celiac disease
- What are the wider implications of this work? “streamline process of getting biologics into clinical trials”
- Oral vs parental and bioavailability discussion
- Why should the reviewers get excited about this? Proof of principle, but what is next step? Long term impact?
- Slide 19- add a box for safety before human studies

TR&D 2:
- Cryogenic grinding for tissue homogenization?
- Put the nanoparticle data in context of bigger picture; consider flashing up the National Academy report on nanoparticles
- Emphasize what you do that is special and unique
- What makes CAMS unique in extraction? With liquid-sample interface, we have sensitivity >100x over anyone else
- Definition of S/N ratio different for different reviewers
- Speakers need to demonstrate more depth on the background for applications
- PAHs: why are you doing it? Not just because you can-

TR&D 3:
- Slide 8: too much detail? Too many weeds to get lost in?
- Slide 13: sample mass= c12?
- Do this talk first for the site visit
- A benefit of this program is that we are contributing to the next generation of multidisciplinary scientists

Remove graphic art elements and any clip art

DBP7
- Gemcitabine-DNA adducts w/ moving wire plot: black on blue is hard to read
- Is there a way to link genetic information to response?
- What your aiming for? Microdose to patients, at some point look at DNA damage in target tissue; translate into prediction of response – aiming at least for a very good negative predictive value
- As you keep applying AMS, eventually you'll have the biomarkers and not need the AMS. Do you see that?

DBP 6
- Slide 8 – hard to read graph axes
- Tables slide: text hard to read
- Need more data
- Why did you choose to look DNA?
- Could you apply this approach to the stem cell tumor hypothesis?
- If you didn't have AMS, could you still do these experiments?
- So far, is this the only quantitative assay that shows promise for assessing metastasis? This is the only system applicable to primary tumor cells. This has the potential to be a unique technology enabled by AMS.
- How much does it cost to do a mouse? AMS: ~100 samples / animals x $100/graphite sample, mouse is ~$100; would be much cheaper w/ moving wire

OUTBRIEF:
- Appalling lack of enthusiasm; get pumped!
- Moving wire is a big deal- need to grasp that and emphasize it
- Rambling, overlapping presentations with too much repetition
- For presentations: Suggest coupling DBPs to TRDs so discussion of DBP science is closely coupled to TRD talks. May set tone for enthusiasm and focus
- Need to present clearer picture that shows exciting possibilities and makes clear we are solving real problems in a unique manner
- Too close to see revolutionary aspects to work? Play up the moving wire data; the fact that this is transformative needs to come through
- Presentations need to be organized so that "here is the problem, this is why it is important and this is why AMS is needed" is very clear. Didn't come through that the coupling was essential
- Every presentation needs to clearly state the unique advantages of AMS to solve the problems at hand
- Make the importance of the problems very clear
- Slides too institutionalized, too sterile

ADMINISTRATIVE INFORMATION

ADMINISTRATION & ALLOCATION OF RESOURCE ACCESS

The Principal Investigator, Kenneth W. Turteltaub, makes Resource allocations with advice from the Internal Executive Committee and the External National Advisory Committee (refer to Advisory Committee pages for list of members). The Internal Executive Committee members are taken from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Lawrence Livermore National Laboratory (LLNL) Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues related to Biomedical AMS. The External National Advisory Committee strives to meet once a year. The Annual Advisory Committee Meeting is focused on the progress being made on the Technology Development projects, the allocation of resources, and the Resource's future. Ad Hoc discussions with individual members of the External National Advisory Committee take place when needed by phone, email or fax.

The Resource makes Biomedical AMS available to the research community through Collaborative and Service subprojects. The operational procedures for access to the Resource are available to the public on the Resource website at http://bioams.llnl.gov/. Prior to formally applying for access to the Resource, an investigator must discuss a proposal concept with an appropriate Resource staff member for evaluation of project compatibility with AMS. Only after pre-screening is an application for access to the Resource submitted to the Resource Administrator. This process has served to eliminate the need to reject applications. New subprojects, which have been accepted into the Resource, are categorized as Collaborative or Service. Collaborative subprojects align with the scientific focus of the Technology Development projects (subprojects 0001, 0002, and 0003) and Resource members play a key role in the project. All other projects are classified as Service. Service subprojects are supported by the Resource for initial feasibility and assisted as needed to ensure successful utilization of AMS in furthering the investigator's studies. Individual Resource staff members provide oversight to all Collaborative and Service subprojects, as they relate to their areas of research interest and expertise.

As a condition for use of the Biomedical AMS Resource, it is expected that investigators will pursue publication of their project's results within one year of successfully completing the studies. When using data in publications and/or presentations, they must acknowledge NIH Resource for Biomedical Accelerator Mass Spectrometry and the supporting NIH/NIGMS grant funding. In compliance with NIH policy, all publications are made publically available. Additionally, copies of preprints for review and release by LLNL and reprints are to be submitted to the Resource Administrator. This past year, work from 25 active subprojects has resulted in 25 publications, of which 3 abstracts, 20 journal articles and 2 book chapters have cited the Resource. (Nine of the publications are in the process of being deposited in PubMed Central).

IACUC AND IRB COMPLIANCE

The Research Resource and its subprojects are in compliance with the Lawrence Livermore National Laboratory's Institutional Animal Care and Use Committee (IACUC) guidelines as well as the Institutional Review Board (IRB) requirements. The requisite approvals are on file and copies of the approval letters are available upon request.

WEBSITE

The Biomedical AMS Resource maintains a website at http://bioams.llnl.gov/. The website was recently refreshed and updated. It now has a section focusing on the liquid sample interface and a training section has been added.

The Resource is also featured on the Physical and Life Sciences Directorate website at https://www-pls.llnl.gov/?url=about_pls-centers_and_institutes-bioams.

AWARDS, HONORS, SPECIAL RECOGNITIONS

SPID 0067:
OSU EMT Research Day Student Platform Award 2014 given to Erin Madeen, trainee
RSESS Student Travel Award to SOT 2013 given to Erin Madeen, trainee
K.C. Donnelly Externship Award (Erin Madeen) from NIEHS ($10,000 direct)

COMMITTEE REPORTS

The External National Advisory Committee met on November 12, 2013. Five of the committee members were in attendance: Tom Baillie, University of Washington; Tom Brenna, Cornell University; Al Burlingame, UCSF; Jim Felton, LLNL retiree; and our newest member, Ileana Cristea from Princeton. A full day of presentations detailing scientific progress of the project and future directions were given by the RR staff, postdocs and students. See the Advisory Committee Section (p. 17) for full details.

DISSEMINATION

POSTERS/ ORAL PRESENTATIONS

Project 2 (SPID 0002)

Avi Thomas, Ben Stewart, Ted Ognibene, Graham Bench, Ken Turteltaub, **Absolute Quantitation of Low-Abundance Protein Adducts using a Novel Accelerator Mass Spectrometry Liquid Sample Interface**, Society of Toxicology meeting, 12 March 2013.

Project 3 (SPID0003)

Paul Henderson, Sisi Wang, Miaoling He, Tzu-yin Lin, Tao Li, Hongyong Zhang, Tianhong Li, David R Gandara, Ken Yoneda, Joyce Lee, Marc Dall'Era, Michael Malfatti, Ralph de Vere White, George Cimino, Kenneth W Turteltaub, Chong-xian Pan, **Phase 0 microdosing trial to determine the feasibility of using drug-DNA adducts as biomarkers of platinum-based drug efficacy**, 246th ACS National Meeting and Exposition, September 8-12, 2013, Indianapolis, Indiana

Paul Henderson, **A Predictive Dx for Cancer Patient Response to Platinum-Based Chemotherapy**, 2014 Personalized Medicine World Conference, Mountain View California, January 27.

Paul Henderson and Amy Pan, **Accelerator Mass Spectrometry to Detect Platinum-induced DNA Adducts and Identify Chemoresistance: from Bench Research to a Phase II Clinical Trial, 2014 Mass Spectrometry: Applications to the Clinical Lab,** March 7, 2014, San Diego CA

H. Enright, P. Nallathamby, V. Mikheev, B. Forsythe, W. Wang, E. Kuhn, S. Retterer, K. Turteltaub, M. Malfatti, **Pharmacokinetics and biodistribution of iron oxide nanoparticles using Accelerator Mass Spectrometry**, Society of Toxicology Annual meeting, March 10-14, 2013, San Antonio, Tx

P. Nallathamby, H. Enright, M. Malfatti, S. Retterer, W. Wang, **Radiolabeled superparamagnetic nanoparticles for bio-distribution studies in life sciences**, Society of Toxicology Annual meeting, March 10-14, 2013, San Antonio, Tx

E. Madeen, R. Corley, K. Turteltaub, T. Ognibene, M. Malfatti, M. Garrard, K. Sudakin, T. McQuistan, D. Williams, ***In vivo* human pharmacokinetics of dibenzo[DEF,P]Chrysene (DBC) following Microdosing—Bridging the gap between high-dose animal data and environmentally-relevant human exposures**, Society of Toxicology Annual meeting, March 10-14, 2013, San Antonio, Tx

#57 (SPID 0048)

Paul Henderson, **An Active Start-up from Lab Research**, Institutional Postdoc Program Board (IPPB) Brown Bag Seminar On Start-ups and Entrepreneurship, Lawrence Livermore National Laboratory, February 6, 2014

#75 (SPID 0068)

Falso MJS, Walsworth V, Buchholz BA, **Transfer of Triclocarban across the Placental Barrier**, Society of Toxicology, March 2012

#84 (SPID 0076)

Maze I, Bagot RC, Wenderski W, Tzavaras N, Noh KM, Sadeh R, Guo Y, Ionete C, Akbarian S, Buchholz BA, Molina H, Nestler EJ, Blitzer RD, Shen L, Allis CD., **H3.3 nucleosomal dynamics regulate synaptic development and plasticity in post-replicative neurons**, Society for Neuroscience in San Diego, CA 2013

Maze I, Wenderski W, Bagot RC, Tzavaras N, Noh KM, Guo Y, Ionete C, Akbarian S, Hurd YL, Tamminga CA, Akbarian S, Buchholz BA, Nestler EJ, Blitzer RD, Shen L, Molina H, Allis CD. **H3.3-dependent nucleosomal dynamics regulate**

synaptic development and plasticity in the central nervous system, New York University, Department of Neuroscience, 1/23/2014

#80 (SPID 0073)

Junyeol Kim, John H. Priester, Yuan Ge, Bruce Buchholz, Elijah Petersen, and Patricia A. Holden, **Trophic transfer of ^{14}C-labeled multi- walled carbon nanotubes in microbial food chains,** UC CEIN Nano EH&S Forum: Scientific Advances Towards Reducing Complexity in Decision Making, Wednesday, May 8, 2013

#35 (SPID 0025)

Kirsty Spalding, Oral Presentation, **Cell and tissue turnover in health and disease, German association for clinical and laboratory medicine**, Dresden, 23-26 October 2013.

Kirsty Spalding, Oral presentation, **Radiocarbon analysis of cell turnover in man**, Vascular Differentiation and Remodeling Meeting, Heidelberg, July 4th, 2013.

Kirsty Spalding, Oral presentation, **Neuronal and adipocyte turnover in adult human brain and fat**, Stem cells and regenerative biology meeting, Jerusalem, Tel Aviv, July 21-25, 2013.

Jonas Frisen, **Neurogenesis in the Adult Human**, Keystone conference on adult neurogenesis, Santa Fe, NM, February 3-8, 2013.

Jonas Frisen, **Neurogenesis in the Adult Human**, Invited seminar, Kings College, London, UK, May 15, 2013

#73 (SPID 0066)

Nima Etminan & Loch Macdonald, **The age of ruptured and unruptured intracranial aneurysms**, Vasospasm meeting, July 10-12, 2013, Lucerne, Switzerland

Nima Etminan & Loch Macdonald, **Determinants for The age of ruptured and unruptured intracranial aneurysms**, International Stroke Conference, Feb. 12-14, 2014, San Diego, USA

#71 (SPID 0064)

Antonio Cannata, Mark Sundman, Sergio Signore, Andrea Sorrentino, Chiara Mangiaracina, Ramaswamy Kannappan, Junghyun Kim, Nicola Alesi, Richard Ke, Polina Goichberg, Barbara Ogorek, Giulia Borghetti, Jan Kajstura, Piero Anversa, Annarosa Leri, and Marcello Rota. **Telomeric Shortening Impairs EC-Coupling and Ventricular Function**. Circulation. 2013;128:A16246.

Anna Czarna, Fumihiro Sanada, Junghyun Kim, Sergio Signiore, Polina Goihberg, Jan Kajstura, Marcello Rota, Piero Anversa, and Annarosa Leri. **Genetic Ablation of c-kit Impairs Cardiac Stem Cell Function**, Leading to Attenuated Cardiomyogenesis and Accelerated Myocardial Aging. Circulation. 2013;128:A17059.

Ewa Wybieralska, Brandon J Elmore, Polina Goichberg, Fumihiro Sanada, Junghyun Kim, Barbara Ogorek, John H Loughran, Emma J Birks, Kelly C McCants, Michael P Flaherty, Marcello Rota, Piero Anversa, Annarosa Leri, Roberto Bolli, and Jan Kajstura. **Clonogenic Human Cardiac Stem Cells With Long Telomeres Can Be Isolated From Minute Endomyocardial Biopsies Circulation**. 2013;128:A15091

Fumihiro Sanada, Junghyun Kim, Anna Czarna, Sergi Signore, Andrea Sorrentino, Chiara Mangiaracina, Antonio Cannata, Kazuya Isobe, Ramaswamy Kannappan, Maria Cimini, Alex Matsuda, Barbara Ogorek, Giorgia Palano, Maria V Caballero, Manila Candiracci, Fiorenza Valeriani, Laura Graciotti, Polina Goihberg, Marcello Rota, Toru hosoda, Jan Kajstura, Piero Anversa, and Annarosa Leri. **Transdifferentiation of Bone Marrow Cells and Cardiomyogenesis in the Infarcted Heart.** Circulation. 2013;128:A15793.

Ramaswamy Kannappan, Giorgia Palano, Polina Goichberg, Fumihiro Sanada, Sergio Signore, Junghyun Kim, Laura Graciotti, Marcello Rota, Jan Kajstura, Piero Anversa, and Annarosa Leri. **p53 Activity Controls Growth and Survival of Cardiac Stem Cells.** Circulation. 2013;128:A17847.

Junghyun Kim, Fumihiro Sanada, Manila Candiracci, Fiorenza Valeriani, Anna Czarna, Antonio Cannata, Polina Goihberg, Toru Hosoda, Jan Kajstura, Marcello Rota, Piero Anversa, and Annarosa Leri. **Genetic Deletion of Telomerase Promotes Premature Cardiac Aging**. Circulation. 2013;128:A18815.

Polina Goichberg, Ramaswamy Kannappan, Maria Cimini, Andrea Sorrentino, Laura Graciotti, Jan Kajstura, Marcello Rota, Piero Anversa, and Annarosa Leri. **Alterations in the EphA2 Endocytic Pathway Interfere With the Motility of Old Human Cardiac Stem Cells**. Circulation. 2013;128:A13292.

#82 (SPID 0077)

A. Daniel McCartt, **Cavity Ring-Down Spectroscopy: Enabling carbon-14 Detection for Biomedical Research**, NIH Headquarters, August 5, 2013

#74 (SPID 0067)

Madeen, E.P., **Human *in vivo* kinetics and dynamics of high molecular weight PAH, dibenzo(*def,p*) chrysene, utilizing liquid sample accelerator mass spectrometry.**, Environmental and Molecular Toxicology Research Day. OSU, Jan 9, 2014

Madeen, E. P.; Corley, R.; Turteltaub, Kenneth W.; Ognibene, T.; Malfatti, M.; Garrard, M.; Sudakin, K.; Mcquistan, T.; Williams, D. E., ***In Vivo* human pharmacokinetics of dibenzo[*def,p*]chrysene (DBC) following microdosing. Bridging the gap between high dose animal data and environmentally relevant human exposures**. Society of Toxicology 52nd Annual Meeting, March 10-14, 2013

E.P. Madeen, R. Corley, K. Turtletaub, T. Ognibene,M. Malfatti, M. Garrard, D. Sudakin, T. McQuistan, D.E. Williams, **Human Pharmacokinetics of the High Molecular Weight Polycyclic Aromatic Hydrocarbon Dibenzo(*def,p*)chysene: Utilizing Sensitive Accelerator Mass Spectrometry to Verify Rodent Model Based Modeling,** Environmental and Molecular Toxicology Research Day. OSU, January 11, 2013

Ken Turteltaub presented:

- “An Overview of Biosciences at LLNL” at KSU, Manhattan KS, 11/2013

Resource Bibliography

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5. Falso M.J.S., Buchholz B.A., (2013) "Bomb pulse biology". ***Nucl. Instr. and Meth. B,*** 294, 666-670. **(SPID 0002)**

6. Ognibene, T.J., and Salazar, G. A., (2013). "Installation of hybrid ion source on the 1-MV LLNL BioAMS spectrometer." ***Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*** 294(0): 311-314. **(SPID 0001)**

7. Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H., Boström, E., Westerlund, I., Buchholz, B.A., Possnert, G., Mash, D., Druid, H. Frisén, J., "Dynamics of hippocampal neurogenesis in adult humans". ***Cell,*** 153 (2013) 1219-1227. **(SPID 0002, 0025)**

8. Salazar, G., and Ognibene, T. (2013) "Design of a secondary ionization target for direct production of a C^- beam from CO_2 pulses for online AMS." ***Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*** 294(0): 300-306. **(SPID 0001)**

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Resource Visitors

The Resource welcomes visitors and has had several during this past year. Visitors have included investigators from the Resource's Collaborative subprojects, potential future collaborators, and groups interested in learning more about Biomedical AMS capabilities. Visitors often gave presentations to the Resource staff on their research, followed by discussions, lab tours and any necessary methodology training.

Visitors to the Center for Accelerator Mass Spectrometry:

Visit date	First Name	Last Name	Affiliation
4/2/13	Craig	Hill	Canberra Industries, Inc.
4/2/13	Paul	Stamets	Fungi Perfecti
4/2/13	Alex	Taylor	Fungi Perfecti
4/8/13	Jason	Henson	International Outreach Services, Inc.
4/8/13	Mackton	Masao	International Outreach Services, Inc.
4/16/13	Simon	Brandt	Liverpool John Moores University
4/16/13	Torsten	Passie	Harvard Medical School
4/18/13	Sam	Kelley	State University @ New York
4/19/13	Taryn	Black	University of Washington
4/19/13	Mika	Usher	University of Washington
4/25/13	Mathias	Stiller	UC Santa Cruz
5/20/13	Leslye	Mohon	Texas A&M
5/22/13	Thomas	Schmidt	USDA Forest Service
6/4/13	Elliott	Campbell	UC Merced
6/4/13	Mary	Whelan	UC Berkeley
6/6/13	Nicole	West	Pennsylvania State University
6/19/13	Ray	Strikas	Center for Disease Control
6/24/13	Lydia	Smith	UC Berkeley
7/18/13	Djordje	Grujic	DALHOUSIE UNIVERSITY
7/24/13	Albert	Carnesale	UCLA
7/24/13	Gary	Was	University of Michigan
7/26/13	Kiersti	Ford	CSU Fresno
7/26/13	Kerry	Workman-Ford	CSU Fresno
8/5/13	Richard	Nishi	Diablo Valley Community College
8/5/13	Sarah	Schrader	Diablo Valley Community College
8/6/13	Sydney	Gunnarson	West Washington University
8/6/13	Sean	Lahusen	Western Washington University
8/19/13	Angelica	Klein	Whittier College
8/30/13	Jack	McFarland	USGS
9/10/13	Andrew	Gray	UC DAvis
9/16/13	Clayton	Williams	Iowa State University
9/19/13	Ana	Morales	Iowa State University
9/24/13	Marie	Champagne	UC Berkeley
9/25/13	Sylvia	Frazier	retired
9/25/13	Bonnie	Rathmell	retired
9/26/13	Wendy	Anderson	Drury University

10/4/13	Ovidiu	Toader	University of Michigan
10/23/13	Rachel	Brown	UC Santa Cruz
11/5/13	Per	Strand	NORWEGIAN RADIATION PROTECTION AUTHORITY
11/7/13	Lea	Braschi	Dalhousie University
11/13/13	Kaila	Stevenson	Mills College
11/14/13	Ben	DeJong	University of Vermont
11/14/13	Danielle	Lemmon	University of Washington
11/15/13	Erin	Madeen	Oregon State University
12/16/13	Prabir	Roy	LBL
1/23/14	Whendee	Silver	UC Berkeley
1/27/14	Paul	deKonkoly-Thege	Williams College
1/31/14	William	Kittle	Pro Temp Mechanics, Inc.
2/19/14	Gene	Henry	Lamont Doherty Earth Observatory
2/28/14	Avriel	Schweinsberg	SUNY-Buffalo
3/1/14	Ian	Desjarlais	CSU Northridge
1/14/14	Lance	Yamaguchi	International Outreach Services, Inc.
1/17/14	Judy	Honda	International Outreach Services, Inc.
1/17/14	Catherine	Murphy	International Outreach Services, Inc. (IOS)
1/27/14	Mea	Cook	Williams College
10/23/13	Ari	Matmon	Hebrew University of Jerusalem
10/24/13	Tobias	Buttersworth Koffman	Lamont Doherty
10/29/13	Sarah	White	UC Santa Cruz
11/12/13	Theodore	Dingemans	University of Nevada/Reno
11/14/13	Guntram	von Kiparski	USGS/Menlo Park
11/14/13	Ray	Weiss	UCSD/SCRIPPS
11/14/13	John	Stone	University of Washington
11/14/13	Gregory	Balco	Berkeley Geochron. Center
11/14/13	Paul	Bierman	University of Vermont
11/15/13	John	Gosse	Dalhousie University
11/18/13	Corey	Lawrence	USGS. Menlo Park
11/4/13	Emily	Lindsey	UC Berkeley
11/7/13	Perry	Spector	University of Washington
12/17/13	Elise	Kali	UC San Francisco
12/23/13	Rachael	Porras	LBL
2/24/14	Kari	Finstad	UC Berkeley
2/27/14	Nicholas	Young	Columbia University
2/27/14	Joseph	Licciardi	University of New Hampshire
2/28/14	Jason	Briner	University of Buffalo

4/18/13	William (Bill)	Jackson	DOE
4/2/13	Steve	LaZar	DOE
4/8/13	Donald	Henry	International Outreach Services, Inc.
5/29/13	Taylor	Broek	UC Santa Cruz
6/14/13	Brittany	Grimm	UC Davis
6/18/13	Chris	Wilkinson Rella	Picarro Instruments
6/19/13	Anthony	Jackson	USDA-APHIS
6/19/13	Erin	Roark	UC Davis
7/24/13	DeWart	Nelson	Simon Fraser University
7/24/13	Guaciara	dos Santos Winston	UC Irvine
7/25/13	Roseanne	Schwartz	Columbia University/Lamont Doherty
8/30/13	Mark	Waldrop	USGS-Menlo Park
8/5/13	Amy	Wagner	Diablo Valley Community College
9/25/13	Robert	Rathmell	Retired

Workshops:

Accelerator mass spectrometry and its applications were featured in a day long workshop, celebrating the 25th anniversary of the founding of the Center for Accelerator Mass Spectrometry

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' #(%!9!' #$%) * !	: ; <=5> ; !, 0- !F0625 - G=6/50!	H; 0253; !) <! 2/. @6A!JKJ !B/2; =652!
' #$%!9!' #7%) * !	D) * E!H, 36A!H2; 3; 06A, 0- !LG6G2; !	M!N!B, O/3A!L5G0- /0. !B/2; =6520D) * E!
' #7%!9!P#((!) * !	EN> 453/G> !) 66; 0- ; ; !B/3=G33/50!	!
P#((!9!P#7%) * !	KG=<, 2!E; =G2/6N!/0!6@ !Q$^{36}!D; 06G2N#! 1@2; , 63QR; 34503; 3!	) <! ; 26!D, 20; 3, <A!D@ 0=; <<52! S> ; 2/6G3A!H25T; 3352A!UDJ) !
P#7%!9!$(#$%) * !	KG=<, 2!L52; 03/=3!EG44526/0. !KG=<, 2!E; =G2/6N!	B, O/- !V/<<A!KG=<, 2!E; =G2/6N! E=/; 0=; 3A!) KE1W!
$(#$%!9!$(#8(!H* !	EN> 453/G> !) 66; 0- ; ; !B/3=G33/50!	!
$(#8(!9!$(#7%!H* !	+2; , X!	!
$(#7%!9!$$#8(!) * !	1@ !U3; !5T!F35654; 3!/0!VG> , 0!V; , <6@!R; 3; , 2=@!	15> !+2; 00, A!H25T; 33352!5T!VG> , 0! KG62/6/50!, 0- !D@ > /362N!Y ! D@ > /=, <!+/5<5. N!A!D520; <<! U0/O; 23/6N!
$$#8(!9!$Q#((!H* !!	1@ !H2, =6/=; !, 0- !6@ !H56; 06/, <!5T!) * E!/0!HGI </=!	Z; 0!1G26; <6, GI A!+/5<5. N!, 0- ! +/56; =@05<5. N!B/O/3/50!J; , - ; 2!
$Q#((!9!$Q#$%!H* !	EN> 453/G> !) 66; 0- ; ; !B/3=G33/50!	
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$#$%!9!Q#((!H* !	1@ !D2/6/=, <!R5< !5T!) ==; < 2, 6; - !F22, - /, 6/50!/0! S03G2/0. !, !LG6G2; !T52!K G=<, 2!S0; 2. N!	? , 2N!: , 3A!: , <6; 2!N/!: ; I ; 2A!M2[! H25T; 3352!5T!EG36, /0, I < !S0; 2. NA! S0O/250> ; 06, <!, 0- !S, 26@!EN36; > 3! S0. [A!U0/O; 23/6N!5T!* /=@/. , 0!
Q#((!9!Q#$%!H* !	EN> 453/G> !) 66; 0- ; ; !B /3=G33/50!	!
Q#$%!9!Q#8(!H* !	+2; , X!	!
Q#8(!9!8#$%!H* !	H25\; =6/503!5T!TG6G2; !D@ 0. ; 3!T25> !? <5I , <! D</> , 6; !* 5- ; <3#!) !J , 2. ; !E=, < !H/=6G2; !5T! U0=; 26, /06N!E5G2=; 3!, 0- !R5I G36!H, 66; 203! S> ; 2. /0. !T25> !D* F!H%!	D< G- /, !1; I , < /A!E2[!E=/; 06/36A! D</> , 6; !D; 062, <!
8#$%!9!7#((!H* !	K 526@ 20!J , 6/6G- ; 3#!D, 2I 50!E;] G; 362, 6/50! E; 2O/=; 3!, 6!R/3X^!	M/!00/T; 2!V , 2- ; 0A!R; 3; , 2=@!E5/<! E=/; 06/36A!U E? EA!* ; 0<5!H, 2X!
7#((!9!7#7%!H* !	V/. @&3; 03/6/O/6N!D53> 5. ; 0/=!K G=</- ; !B , 6/0. !, 0- ! /63!F> 4, =6!50!S, 26@!EG2T, =; !E=/; 0=; !	M3!; 2. !E=@ ; T; 2A!J , > 5068!35@ 26N! S, 26@!W I 3; 2O, 652NA!) - \G0=6!) 335=[! H25T; 3352A!D5<G> I /, !U0/O; 23/6N!
7#7%!9!%#((!H* !	EN> 453/G> !) 66; 0- ; ; !B /3=G33/50!	!
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Classification Level: Unclassified
Agenda Date: July 9, 2013
Property Protection Area. Foreign national temporary escorted building access procedures apply.

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PATENTS, LICENSES, INVENTIONS AND COPYRIGHTS:

US008642953B2

(12) **United States Patent**
Turteltaub et al.

(10) **Patent No.: US 8,642,953 B2**
(45) **Date of Patent: Feb. 4, 2014**

(54) **INTERFACE FOR THE RAPID ANALYSIS OF LIQUID SAMPLES BY ACCELERATOR MASS SPECTROMETRY**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/396,461**

(22) Filed: **Feb. 14, 2012**

(65) **Prior Publication Data**

US 2012/0235031 A1 Sep. 20, 2012

Related U.S. Application Data

(60) Provisional application No. 61/452,915, filed on Mar. 15, 2011.

(51) **Int. Cl.**
H01J 49/04 (2006.01)

(52) **U.S. Cl.**
USPC **250/288**; 250/282

(58) **Field of Classification Search**
USPC 250/281, 282, 288
See application file for complete search history.

(56) **References Cited**

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Sessions et al, "Moving-Wire Device for Carbon Isotopic Analyses of Nanogram Quantities of Nonvolatile Organic Carbon", Anal. Chem, 2005, 77, 6519-6527.*

(Continued)

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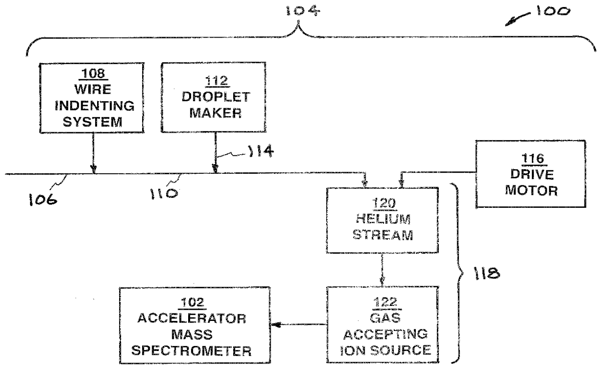
(57) **ABSTRACT**

An interface for the analysis of liquid sample having carbon content by an accelerator mass spectrometer including a wire, defects on the wire, a system for moving the wire, a droplet maker for producing droplets of the liquid sample and placing the droplets of the liquid sample on the wire in the defects, a system that converts the carbon content of the droplets of the liquid sample to carbon dioxide gas in a helium stream, and a gas-accepting ion source connected to the accelerator mass spectrometer that receives the carbon dioxide gas of the sample in a helium stream and introduces the carbon dioxide gas of the sample into the accelerator mass spectrometer.

2 Claims, 16 Drawing Sheets


104
100
108 WIRE INDENTING SYSTEM
112 DROPLET MAKER
114
116 DRIVE MOTOR
106
110
120 HELIUM STREAM
118
102 ACCELERATOR MASS SPECTROMETER
122 GAS ACCEPTING ION SOURCE

TRAINING:

The Resource’s training manual and associated test continue to be a key tool, used to train investigators working in the Resource’s laboratories and to verify their knowledge of the policies, practice and procedures. The training set and test have been recently updated and are now included on the center’s website: **http://bioams.llnl.gov/**.

Four Ph.D. Students are associated with Resource Projects:

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- Junyeol Kim, UCSB, SPID0076

Six Postdocs are associated with the Resource:

- **Heather Enright**, toxicology
- **Sean Gilmore**, funded by a LLNL-UC Davis Cancer Center Fitzpatrick Fellowship, cancer therapeutics
- **Windy McNerney**, neurobiology
- **Hirmoi Wettersten**, funded by a LLNL-UCDCC Fitzpatrick Fellowship, tumor metabolism
- **Tiffany Scharadin**, UC Davis, drug resistance
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SUBPROJECT DESCRIPTIONS

The following changes in subprojects have occurred since our previous progress report:

SPID 0019 has been determined to be inactive

SPID 0032 is complete

SPID 0053 is complete

SPID 0055 is complete

SPID 0057 has been determined to be inactive

SPID 0062 is complete

SPID 0072 has been determined to be inactive

SPID 0025 Cell turnover in adults using a novel method for the retrospective birth dating of cells (C)

RR project # 35

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PROJECT DESCRIPTION:
Neurogenesis is known to occur in specific regions of the adult animal brain, but the extent and comparability of neurogenesis in the adult human brain is much harder to determine, and to date largely unknown. Traditional methods used for dating cells are limited in the

information they provide, or are not appropriate for human use. Thus, currently there is no method available to study cellular turnover in man. We propose to develop a method for the retrospective birth dating of cells. We are interested in using bomb pulse carbon-14 (C14) dating as a method for measuring the approximate age of specific populations of cells in the adult human brain. This method is based on establishing the proportion of the isotope C14 in genomic DNA. C14 measurements will be made using the highly sensitive accelerator mass spectrometer (AMS).

After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA will reflect the age when the cell was born. Traditionally, the slow decay of C14 relative to other carbon species has given it a temporal resolution of many years, however due to nuclear tests in the late 1950s and early 1960s, the level of C14 in the atmosphere has increased dramatically. This level has since dropped off in an exponential fashion, allowing one to resolve C14 differences in the range of years. Because DNA has a C14 content reflective of the time when it was synthesized, establishing the C14 content of chromosomal DNA will enable us to retrospectively birth date cells, and thus establish cellular turnover.

Crucial to the understanding of basic biological processes, is information about cellular turnover. As well as having an interest in normal cellular turnover, many diseases are thought to be affected in their generation of new cells. Information about cellular turnover in disease states may provide novel insights into the pathological processes of the disease, and possibly suggest new therapeutic strategies. Particular populations of cells will then be isolated using FACS analysis (which allows specific cell populations to be isolated e.g. one can sort for neurons using neuronal specific markers such as NeuN). The technique has also been applied to cardiomyocytes and adipocytes. We are investigating the turnover of lipids within adipocytes also. The use of enamel as a forensic tool to establish date of birth has been under investigation the past couple years.

Research Progress (through 3/31/2014):

A paper on hippocampal neurogenesis was published.

A paper examining teeth from North America and comparing to those from other global locations was published.

SPID 0027 Identification and Development of Biological Markers of Human Exposure to the Insecticide Permethrin (C)

RR project # 37

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PROJECT DESCRIPTION:

Agricultural workers, gardeners and homeowners are routinely exposed to the insecticide permethrin. Also, military personnel are exposed to the permethrin when using the DOD Insect Repellent System and over-the-counter lice soaps use permethrin as the active ingredient. A urinary metabolite of permethrin, that is in high abundance and is relatively stable, may be an ideal biomarker of exposure to this pesticide. In addition, the ratio of one metabolite to another may vary, according to the route of administration. The results of this study would be used to identify candidates for the development of a rapid, sensitive immunochemical based analytical method that can be used to routinely monitor human exposure to permethrin.

Objectives: The purpose of this study is to determine the human metabolite(s) of permethrin in urine following dermal exposure that are in greatest abundance and are the most stable. Accelerator mass spectrometry is a method for measuring levels of 14C several orders of magnitude more sensitive than liquid scintillation counting. With this high sensitivity we will conduct human metabolism studies at biologically relevant doses.

SPECIFIC AIMS: I. Develop an LC/MS method for separation of permethrin and its putative human metabolites. II. Determine the human metabolite profile of permethrin using accelerator mass spectrometry (AMS). III. Develop an immunoassay to the key metabolite identified in Objective II as a biomarker of human exposure to permethrin.

METHODOLOGY: For specific aim I, synthesize metabolite standards; develop an HPLC method to separate the putative pyrethroid metabolites using ultraviolet detection; determine the feasibility of an HPLC/mass spectrometry method for analysis of pyrethroid metabolites. For specific aim II, clinical exposure of humans to radiolabeled permethrin dermally and collection of urine, blood and saliva; separation of samples by methods developed in specific aim I and analysis of separated samples by accelerator mass spectrometry; identification of most prevalent metabolite from resultant data. For specific aim III, synthesis of haptens; development of antibodies; use of the haptens and antibodies in the development of an immunoassay for the most prevalent metabolite; validation of immunoassay.

EXPECTED PRODUCTS (MILESTONES): Literature review of putative human metabolites of permethrin; small quantities of synthesized metabolites of permethrin; an HPLC method for separating permethrin metabolites in human urine or saliva; identification of the most abundant human metabolite(s) by accelerator mass spectrometry; an immunoassay to detect the targeted human metabolite of permethrin

STATUS/RESULTS TO DATE: Literature review has been completed and putative major metabolites identified. All of the major metabolites have been synthesized or acquired. Using the metabolite standards a high performance liquid chromatography method for their analysis has been developed. This method will later be used to identify metabolites found in human urine samples and the liquid chromatography-mass spectrophotometric method used for validation. Using the chemical knowledge from the preparation of metabolites, synthesis of haptens for immunoassay detection of these molecules is complete. Immunoassays for 3-phenoxybenzoic acid, the glycine conjugate of 3-phenoxybenzoic acid, the glycine conjugate of dichlorovinylchrysanthemic acid (DCCA) and the glucuronide conjugate of 3-phenoxybenzyl alcohol (see publication below) have been developed. An assay for free DCCA is in progress (manuscript in preparation). The assay for 3-phenoxybenzoic acid has been adapted to a sensitive, high throughput method (see publication below). Clinical exposures have been completed. All samples have been measured by AMS for total carbon-14. An estimate of the total dose absorbed (for 4 subjects) ranged from 0.06 to 0.27%. The permethrin is eliminated from the blood with a half life of about 12-24 hours hours. The urinary half life averaged 24 hours. Saliva was sampled, but permethrin does not appear to be excreted by that route. Liquid chromatography analysis of the metabolite pattern in urine is underway.

Conclusion: The results of this study will be used to identify candidates for the development of a rapid, sensitive immunochemical based analytical method that can be used to routinely monitor human exposure to permethrin. The ability to carefully monitor the presence of absorbed doses of permethrin will be a useful tool to prevent the possibility of human health effects due to permethrin exposure.

Research Progress (through 3/31/2014):
Manuscript is in preparation.

SPID 0038 Immunochemical Methods to Monitor Toxic Substances and/or Indicators of their Presence in Humans and Other Species (C)

RR project # 8

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PROJECT DESCRIPTION:
Anthropogenic environmental toxins, even at low doses, cause some measure of biological change to take place, within plants, animals, microbes, or even humans. The goal of the UC Davis Superfund Program is to discover ways to observe and quantify these biomarkers of environmental impacts, so that the sources and causes of these impacts can be understood, assessed, traced, and remediated. To that end, the Program includes AMS quantitation as one of its competencies in its analytical core. This analytical core serves several of the Program projects, including Soil and Waste Transport, development of Immunochemical Biomarkers, Pulmonary Biomarkers, and Reproductive Biomarkers.

Accelerator mass spectrometry (AMS) plays an important role in the assessment of human exposure to toxic substances and in probing the mechanistic basis of toxicity in humans and in other host species. It is a core technology in our program of using biomarkers of environmental exposures to toxic substances from agricultural and industrial activities. We define urinary, pulmonary, reproductive, and circulating biomarkers of specific toxic exposures that are quantifiable using assays such as immunoassays, protein mass spectrometry, chromatography, and direct quantitation of isotope labeled toxins with AMS. AMS also provides calibration of the other assays through correlation of isotope label incorporation from toxins into a host. Quantitation of a derived biomarker is then calibrated by the uptake of toxin indicated by the AMS measurements.

In the case of Transport, the investigators are assessing the biological activity of the recently used fuel additive, methyl-tert-butyl-ether (MTBE), which leaked into the ground from fuel depots over the past decade. The binding of 14C-MTBE to mammalian protein is being studied to determine if the compound presents a threat to cellular systems. These laboratory studies are freely done with the levels of 14C needed to interact with cell cultures, but much of the Program is concerned with quantifying biomarkers in natural settings where radiotracer release is not possible. The preferred technology or quantifying recognizable biomarkers is the immunoassay which can eventually be made into field-usable kits. It is important to choose the right target for immunoassay development, such as the most likely metabolite or hormonal response of a chemical exposure. AMS is a particularly valuable technology for the discovery of optimal immunoassay targets because it reveals all metabolites of an isotope-labeled xenobiotic, even at low dose exposures. We found that the di-dealkyl mercapturate metabolites of atrazine were the most prominent lasting biomarkers of this ubiquitous herbicide in humans. Additionally, although 3-phenoxybenzoic acid is commonly used as a biomarker of exposure to pyrethroid insecticides, a conjugated form predominates. Immunoassays are developed for these biomarkers.

There are "marker" species in ecosystems which are sensitive to environmental change, much like the canaries of past centuries in coal mines. An increasing number of pollutants are being seen as hormonal mimics that act as "poison" to a species by impairing its reproductive success. We are using small quail as one such example and are finding the metabolites of testosterone or cortisone in their fecal droppings, which are used as sample so as to avoid stress effects in a captured bird. The pattern of metabolites will be quantified to find which might be signs of slowly developing environmental stresses. The birds are small, and cannot be heavily dosed, so the sensitivity of AMS is needed.

Pulmonary responses to environmental chemicals need to be studied from respiration of environmentally relevant doses. The dose deposition in specific proteins of lung tissue of model animals is poorly quantified by present methods that provide a large exposure followed by protein separation on two-dimensional gels followed by long term (1 month) autoradiography. AMS has the sensitivity for appropriate doses and sequential gel separations have been worked out to maximize target protein discovery.

The AMS core serves to identify prominent biomarkers of exposure for fieldable assay development and quantifies exposures to labeled compounds for the Program researchers.

Research Progress (through 3/31/2014):
Two other RR projects received ^{14}C-labeled compounds from RR08. Transport of ^{14}C-TCC Across the Placental Barrier (RR75) received ^{14}C-TCC(triclocarban). AMS to Determine [^{14}C]Tetramethylenedisulfotetramine Binding to GABAA Receptor (RR86) received the labeled rodenticide14C-TETS (tetramine).

Prof. Alan Buckpitt and Dr. Buchholz (LLNL) have conducted initial ex vivo exposures of mouse and rat respiratory tissues to naphthalene (NA) in search of DNA-NA adducts. NA forms protein adducts abundantly. Initial work has measured DNA-NA adducts in tissues that produced tumors after chronic exposures to NA. Work is being done to ensure DNA separations are free of protein that could give erroneous results. A proposal was submitted to the DOD Lung Cancer Research Program on DNA-NA adducts.

SPID 0043 B12 Absorption Kinetics and Transcobalamin Genotype (C)

RR project # 51

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PROJECT DESCRIPTION:

The overall goal of our research is to utilize carbon-14 labeled vitamin B12 (14C-B12) and accelerator mass spectrometry (AMS) to assess the absorption and turnover of vitamin B12 in humans, as well as the bioavailability of vitamin B12 from foods. With respect to absorption and turnover, we have dosed 6 healthy control subjects with 14C-B12 (50 nCi, 1.3 μg) and collected baseline and post-dose blood, urine, and stool samples for assessment of 14C-B12 by AMS. The data show relatively consistent patterns of absorption and turnover among the subjects, including first appearance of 14C-B12 in the plasma at about 3 hours, peak plasma levels between 6 and 8 hours, followed by similar plasma elimination curves. A surprising finding has been our observation of high levels of 14C in the urine (30-40% of the administered dose), which is in direct contrast to previous literature reports of 0.1-0.5% of the administered dose in studies using 57Co-labeled vitamin B12. We have hypothesized that we are observing breakdown of the vitamin B12 in the gastrointestinal track that was not possible to observe when using 57Co-B12. This finding has potential implications for the bioavailability of B12 from pills and from foods to which vitamin B12 has been added as a fortificant. Future studies will focus on determining if 14C-B12 can be used to diagnose vitamin B12 malabsorption syndromes (e.g. pernicious anemia) and developing a model for vitamin B12 absorption and turnover in humans. In addition, we will attempt to determine how vitamin B12 is broken down in the gastrointestinal tract and what are the products of this breakdown that seem to be appearing in the urine.

For assessment of vitamin B12 bioavailability from foods, we are enriching chicken eggs *in vivo* with radioactively labeled vitamin B12 to a level that allows us to feed the enriched eggs to humans and determine how much of the vitamin B12 is digested and absorbed into the body. The results of our first experiment indicate that we can inject radioactively labeled vitamin B12 into a laying hen and detect the radioactive vitamin B12 in the eggs at a level sufficient for feeding to humans in a bioavailability study. The *in vivo* labeled eggs have been prepared for human consumption and a single dose of ~20 nCi in labeled scrambled egg was fed to a human subject. We were able to track the appearance of ^{14}C-B12 in plasma, urine and feces at this level of enrichment. We are currently recruiting additional subjects for further study.

SPID 0048 Developing a new AMS Assay for Quantitation of Platinum-DNA Adducts and Predictive Value of DNA Adducts for Response to Platinum-based Chemotherapy (C)

RR project # 57

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PROJECT DESCRIPTION: AMS will be applied to measuring the platinum-DNA adducts for drug pharmacokinetics. In spite of the importance of platinum-based anticancer drugs (cisplatin, carboplatin and oxaliplatin), their mechanisms of action, repair of damaged DNA and pharmacokinetics are unclear because of the detection limit of conventional methods (conventional methods have failed in quantifying Pt-DNA adducts with cells incubated with a pharmacological dose of the anticancer agent, which is the reason we need the sensitivity of AMS). In order to address these important issues, 14C-labeled carboplatin and oxaliplatin will be administered to E. coli, human cells, and bladder cancer patients, which may overcome the previous detection limits even at sub-pharmacological doses. The goals are to use AMS to elucidate their in vivo mechanism of action, to correlate Pt-DNA adduct level with cell death using a variety of human cancer cells, to determine the pharmacokinetics of the patients dosed with 14C-labeled carboplatin and oxaliplatin, and to ultimately correlate the phamacokinetic results to individual outcome (patient survival). Experimentally, after dosing a number of human cancer cells or cancer patients with radioactive platinum-based anticancer drugs, cell lysis and extracted platinated DNA will be measured by AMS. These 'real-time pharmacokinetics' will allow determination of which cancer patients will benefit from platinum treatment and which will be resistant to the drugs. Because of the high sensitivity, AMS is the very best technology for realizing these challenging goals.

**Research Progress (through 3/31/2014): A UC Davis clinical study aimed at developing a test to predict response to platinum-based chemotherapy was expanded to include three additional sites: UCSF, UCLA and USC. To date sixteen patients have been accrued to the study. All of the patients were given a microdose of carboplatin that was radiocarbon labeled. Blood and tumor biopsy samples were taken within 24h after microdosing. Within four weeks patients began platinum-based chemotherapy and were monitored for objective responses including reduction in tumor volume, progression-free survival and overall survival. Response data are starting to be acquired, and the expected trend that the highest microdose-induced drug-DNA adduct levels correlate to response to therapy was observed. However, the trend is not yet statistically significant and we need to accrue additional patients. Over the last year we made progress getting IRB approval for the study and UCSF, UCLA and USC, but are still waiting to accrue patients at those sites. We recently initiated a similar clinical study, but with the focus on a different drug, oxaliplatin, in the breast cancer setting. We manufactured under GMP the [^{14}C]oxaliplatin dosing solutions and obtained IRB approval over the last year. The study is now open to accrual.

SPID 0054 Assessment of Vitamin B12 Bioavailability from Eggs and Bread (C)

RR project # 62

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PROJECT DESCRIPTION:
Vitamin B12 (B12) is an essential nutrient required for the normal functioning of mammalian cells, particularly in the hematological and neurological systems. All naturally occurring B12 is synthesized exclusively by bacteria since both plants and animals lack the necessary enzymes for the biosynthesis of B12. Intestinal bacteria synthesize B12, which is absorbed and incorporated into animal tissues, as well as milk and eggs, making these products primary dietary sources of B12 for humans. However, there is only limited information available on the bioavailability of B12 from these dietary sources. A major technological advance, accelerator mass spectrometry (AMS),

available through collaborative arrangements with the Lawrence Livermore National Laboratories, provides the capacity to detect trace levels of carbon-14 (^{14}C) in biological samples at attomolar (10^{-18}) concentrations. The instrument is therefore uniquely suited for assessing the biological fates of ^{14}C-labeled substances after oral ingestion. In ongoing NIH-sponsored human studies, we are assessing the absorption, metabolism, and turnover of a single oral dose of ^{14}C-labeled B12 (^{14}C-B12) dissolved in water. In a pilot feasibility study funded by the National Cattlemen's Beef Association (NCBA), we have successfully enriched beef muscle and liver *in vivo* with ^{14}C-B12. The goal of this proposal is to enrich chicken eggs *in vivo* with ^{14}C-B12 and feed the ^{14}C-B12-enriched eggs to healthy human subjects to determine B12 bioavailability from eggs. Eggs and dairy products are the only non-meat sources of vitamin B12.

Eggs

Chicken eggs enriched *in vivo* with ^{14}C-B12 were fed to human volunteers. Baseline and post-ingestion blood, urine and stool samples were collected over a one-week period and assessed for ^{14}C-B12 content using accelerator mass spectrometry. Bioavailability is being determined by measurement of the fraction of the total oral dose appearing in the stool, as well as calculation of the area under the curve (AUC) reflecting the appearance and disappearance of ^{14}C-B12 from plasma. The hypothesis to be tested is that an accurate value for the bioavailability of B12 from chicken eggs can be determined, which in turn can allow us to definitively determine the nutritional value of eggs as a source of B12. Data is currently being analyzed.

Bread

Cobalamin (vitamin B12) deficiency is highly prevalent in the US and worldwide. Deficiency is most common in the elderly, with an average prevalence ≈25% over age 60 y. The situation is similar in California; in a representative sample of 1545 Latinos aged ≥60 y in the Sacramento region, we observed 6.5% deficiency and an additional 16% marginal status. About 40% of older persons with low serum cobalamin have food cobalamin malabsorption (F-CM), in which gastric atrophy and/or dysfunction is implicated. The gastric atrophy is often the result of chronic infection with Helicobacter pylori, which over time causes hypochlorhydria and diminished production of gastric pepsin, followed by loss of mucosal integrity, and subsequent overgrowth of bacteria in the stomach and upper intestine. The hypochlorhydria and failure of pepsin production impairs release of cobalamin from proteins in food, and the bacterial overgrowth may compete for uptake of cobalamin.

Most elderly with F-CM can still absorb synthetic, crystalline forms of the vitamin when added to fortified cereals or used as supplements. It is generally recommended that elderly consume a higher proportion of their cobalamin in fortified foods than younger people. However there is considerable debate about whether crystalline cobalamin is absorbed as well by elderly with F-CM, especially if added as a fortificant to food. Elderly in general seem to need substantially higher intakes than the RDA to restore or maintain their cobalamin status, even when the vitamin is given as a supplement. The question of whether the most at-risk elderly, those with gastric atrophy, can absorb vitamin cobalamin added as a fortificant to cereals is especially timely based on recent reports that those with deficiency but high serum folate have five times the risk of cognitive impairment and anemia compared to those with normal cobalamin and folate status. Another problem is that when low serum cobalamin is diagnosed in older people, it is often ignored by medical practitioners until depletion progresses further, or there are symptoms of deficiency (such as nerve damage). Alternatively high oral doses, or injections, are prescribed for the rest of life. Diagnosis of F-CM is rarely attempted. It has not yet been investigated whether treating the Helicobacter pylori and bacterial overgrowth, achievable with the same medications, will improve ability to absorb cobalamin from food or in the crystalline form.

Our hypothese is: Older adults with markers of gastric atrophy will absorb less ^{14}C-cobalamin added as a fortificant to bread than will older adults with no sign of gastric atrophy. The research will employ a novel method to assess absorption using 14C-cobalamin that has very low levels of radioactivity which will be quantified by accelerator mass spectrometry. The ^{14}C-cobalamin will be added to bread as a fortificant, at a level similar to that which might be added in flour fortification. Persons aged ≥60 y will be screened to identify those with low cobalamin, who will then be assessed for serum markers of gastric atrophy. Ten with evidence of gastric atrophy, and ten without, will consume the labeled bread, and absorption of cobalamin compared by quantifying radioactivity in plasma and urine during the next 24 h.

Research Progress (through 3/31/2014):

A single paper for the Egg project including the ^{14}C-B12 *in vivo* labeling of eggs and the bioavailability of ^{14}C-B12 *in vivo* labeled egg in humans is being written. The bread B12 project is being summarized in a short report. .

SPID 0059 Carbon-14 based age analysis of metastatic prostate cancer samples (C)

RR project # 67

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PROJECT DESCRIPTION:
We have previously shown that genomic copy number analysis of multiple metastases from men with metastatic prostate cancer can be used to distinguish clonal from nonclonal genomic changes. Recent carbon-14 based studies have proven valuable in establishing the rate of cell turnover in myocardium and other tissues. The primary aim of a carbon-14 based study of metastatic prostate cancer DNA is to establish the relative age of various metastases in a given individual with metastatic prostate cancer, to provide valuable new insight into the natural history of metastatic cancer lesions.

The scientific purpose is to understand the natural history of metastatic cancer, so that diagnostic methods and treatment can be better tailored to each person with metastatic cancer. Do all metastases in an individual have a similar profile, or are they measurably different in age, and do these differences in age correspond to differences in clonal and subclonal copy number changes? Genomic DNA samples for the study are from the Johns Hopkins Integrated Clinical –Genomic Autopsy Study of Lethal Prostate Cancer. DNA samples studied include at least three anatomically separate metastatic sites and at least one noncancerous DNA sample from up to 12 subjects. The AMS technique is the only method with sufficient accuracy and precision to measure bomb-pulse carbon-14 in relatively small DNA samples.

SPID 0061 Analysis of Cold War C14 Levels in DNA from Human Prostate Tissues (C)

RR project # 68

Investigators:

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PROJECT DESCRIPTION:

Introduction: Prostate diseases such as prostate cancer and prostatic enlargement (i.e. benign prostatic hyperplasia, or BPH) are major health problems among American men. The growth of the prostate gland occurs in various stages throughout the lifespan of male individuals, and both prostate cancer and BPH are the result of aberrant cellular turnover and growth. However, this process of cellular turnover is very poorly understood, particularly in humans. The goal of this project is to use AMS to measure Carbon-14 levels in the DNA of prostate cells as a result of Cold War atomic bomb testing. Measurement of Carbon-14 in the post-bomb testing era allows an accurate assessment of tissue turnover and cellular birth-dates; such an approach allows important questions in prostate biology research to be answered regarding the etiology of prostate disease.

Methods: DNA will be extracted from tissue specimens, quality controlled, and submitted for Carbon-14 analyses using AMS. Tissue specimens will included archived tissue specimens from our institution (1955 – present), as well as fresh prostate tissue specimens from patients currently at our institution. Carbon-14/Carbon-12 ratios will be compared to the known atmospheric Carbon-14 levels, as previously reported, and tissue and cellular "dates of birth" will be determined.

Anticipated Results: We anticipate that AMS will accurately determine the rate of turnover within the prostate cellular compartments, and that such Carbon-14 levels will indicate the time of disease development when compared between normal prostate, prostate cancers, and BPH. Furthermore, such an approach will document the average lifespan of the various cell types within the prostate gland, such as the long-lived prostate stem cell. Finally, data generated from these studies will greatly facilitate the generation of new hypotheses regarding prostate function and disease etiology.

Impact: Data generated from these studies will have an immediate impact on the field of prostate cancer research and will facilitate answers to essential - and as yet unanswered - questions pertaining to prostate growth, maintenance, and the etiology of prostate disease.

Research Progress (through 3/31/2014): We have prepared a manuscript and are discussing additional experimental approaches to elucidate the role of formalin fixation and paraffin embedding on contaminating carbon within human prostate tissues.

Dr. Vander Griend was awarded an RO1 (1R01CA178431 - 01A1 PI Name: Vander Griend; pending start date 3/1/14) on his work with stem cells and prostate cancer. This will facilitate Dr. Vander Griend's tissue collection protocol which will positively impact his work on tissue turnover and C14 measurements.

SPID 0064 Turnover of Cells in the Human Myocardium (C)

RR project # 71

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PROJECT DESCRIPTION:

1) Redefining human myocardial biology.

For nearly 4 decades, the human heart has been considered a post-mitotic organ composed of a predetermined number of myocytes, which is established at the end of gestation. According to this Old Paradigm, the generation of myocytes ceases at birth and their number is preserved throughout life until death of the organ and organism. Cardiac growth postnatally and organ hypertrophy in the adult occur only by myocyte enlargement. On this premise, the age of myocytes corresponds to the age of the organ and organism, i.e., cellular, organ and organism age coincide. Recent results from our laboratory and others have documented that tissue specific stem cells reside in the human heart. Human cardiac stem cells (hCSCs) are self-renewing and multipotent in vitro and in vivo; hCSCs differentiate in myocytes, and vascular smooth muscle cells (SMCs) and endothelial cells (ECs) organized in coronary vessels. The recognition that the human heart possesses a stem cell compartment has imposed a reevaluation of cardiac homeostasis, aging and pathology. The New Paradigm refutes the conviction that myocytes are formed only during embryonic development and suggests that the replacement of coronary vascular SMCs and ECs is regulated by differentiation of hCSCs rather than by the ability of these mature cells to divide. A novel conceptual framework of the heart has emerged; the Heart Is a Self-renewing Organ characterized by a compartment of resident stem cells. This discovery has laid the ground work for the use of hCSCs in the treatment of the failing heart. Currently, two phase I clinical trials are in progress (ClinicalTrials.gov Identifier: NCT00474461; Identifier: NCT00893360).

Our understanding of the cellular processes implicated in the maturation, homeostasis and repair of the human heart is extremely deficient and the need for basic information is striking. Findings in nematodes, fruit flies, zebrafish and rodents have often been translated to human beings with little caution, emphasizing the necessity to study the fundamental principles that regulate the plasticity of the myocardium during the lifespan of women and men. Moreover, the mechanisms modulating the response of the female and male heart to ischemic and non-ischemic myocardial injury and the principal factors conditioning end-stage heart failure and death in humans are at present unknown. Thus, the major objective of this application is to establish the rate of myocyte and non-myocyte turnover mediated by hCSC activation and differentiation in the developing, adult, aging and failing heart. To achieve this goal, we will employ retrospective ^{14}C birth dating of cardiac cells to establish the average age of myocytes and non-myocytes. This information will be complemented by defining the age distribution of myocytes and non-myocytes utilizing a mathematical model of age-structured cell populations. These data will offer a novel comprehensive perspective of the cellular processes which govern the lifespan of the human heart. This information is critical for the recognition of the mechanisms that control the dynamics of the human heart, its growth reserve, adaptation to stress and failure.

2) Aging of the heart

Currently, we have little understanding of the etiology of myocardial aging. Rarely, studies in animals and humans have considered aging as an independent process and time as the major cause of the aging myopathy. Only occasionally cardiac aging in humans has been characterized independently from concomitant pathologic states. Aging has been interpreted as a variable, which cooperates with a variety of diseases, to define the old, poorly functional heart. This Program Project Grant (PPG) aims at the: a) Definition of myocardial aging; b) Identification of the determinants of the cardiac senescent phenotype; and c) Recognition whether the aging myopathy conditions health and life span. The major hypothesis to be tested is that aging of cardiac stem cells (CSCs) affects the size and properties of the myocyte, vascular, and fibroblast progeny which, in turn, conditions the structure and function of the heart. Aged CSCs may generate a smaller number of senescent myocytes with defects in electrical and mechanical behavior, and a larger number of fibroblasts which, together, underlie diastolic dysfunction and the old cardiac phenotype.

This PPG has five objectives: a) To determine whether the adult heart is a self-autonomous organ regulated by the orderly organization and growth of CSCs; b) To determine whether telomeric shortening in CSCs with aging leads to time-dependent changes in the growth properties of CSCs and characteristics of the differentiated progeny; c) To determine whether old CSCs with short telomeres generate functionally-defective myocytes together with enhanced formation of fibroblasts; d) To determine whether accumulation of fibroblasts and myocytes with impaired contractile performance promotes diastolic dysfunction, typically present in the senescent heart; and e) To determine whether strategies preventing the aging myopathy, or reversing myocardial aging can be developed to extend health and life span in the elderly.

The common theme of this PPG is understanding the control of CSC growth and commitment, the etiology of CSC senescence and death, and the impact that old CSCs have on the properties of the differentiated progeny. The telomere-telomerase axis is viewed as the key regulator of CSC replication, senescence and death, conditioning myocyte, coronary vasculature, and organ aging. Alterations in the turnover rate of cardiomyocytes, vascular cells, and fibroblasts define the aging myopathy. CSCs with preserved function are present in the old heart, and repopulating protocols with CSCs possessing intact telomeres may replace defective myocytes with new, mechanically efficient cells, and restore the coronary vasculature and microvasculature reversing the senescent phenotype, ultimately, prolonging health and life span of the organ and organism. To fulfill these objectives, the role of CSCs in myocardial aging of small (Projects 1 and 2) and large (Project 3) animals and humans (Project 4) will be investigated in an integrated manner to identify the variables that lead to ventricular dysfunction in the old heart.

Research Progress (through 3/31/2014):

During the last funding period, the major activity ongoing in the laboratory consisted of the execution of all experimental assays required to carry out the studies outlined in the two proposals. Importantly, a major effort was made to gain insights, knowledge, and clear understanding of two key concepts advanced in this application: a) the aging myopathy is a stem cell disease; and b) alterations in the telomerase-telomere system is the critical determinant of cardiac aging and diseases.

Significant results.
a) Aging negatively impacts on the function of resident human cardiac progenitor cells (hCPCs). Effective regeneration of the injured heart requires mobilization of hCPCs to the sites of damage. In the young heart, signaling by the guidance receptor EphA2 in response to the ephrin A1 ligand promotes hCPC motility and improves cardiac recovery after infarction. We report that old hCPCs are characterized by cell-autonomous inhibition of their migratory ability ex vivo and impaired translocation in vivo in the damaged heart. EphA2 expression was not decreased in old hCPCs; however, the elevated level of reactive oxygen species in aged cells induced post-translational modifications of the EphA2 protein. EphA2 oxidation interfered with ephrin A1-stimulated receptor auto-phosphorylation, activation of Src family kinases, and caveolin-1-mediated internalization of the receptor. Cellular aging altered the EphA2 endocytic route, affecting the maturation of EphA2-containing endosomes and causing premature signal termination. Overexpression of functionally intact EphA2 in old hCPCs corrected the defects in endocytosis and downstream signaling, enhancing cell motility. Based on the ability of phenotypically young hCPCs to respond efficiently to ephrin A1, we developed a novel methodology for the prospective isolation of live hCPCs with preserved migratory capacity and growth reserve. Our data demonstrate that the ephrin A1/EphA2 pathway may serve as a target to facilitate trafficking of hCPCs in the

senescent myocardium. Importantly, EphA2 receptor function can be implemented for the selection of hCPCs with high therapeutic potential, a clinically relevant strategy that does not require genetic manipulation of stem cells. As proposed in the original application, this information is fundamental for the documentation that the aging myopathy is a stem cell disease and for the potential implementation of stem cell therapy in human myocardial aging and heart failure.

b) Despite the identification of cardiac stem cells and their ability to differentiate in the various cardiac cell lineages, the magnitude of the turnover rate of myocytes in the adult heart remains to be elucidated. Two independent but complementary protocols were employed to define myocyte renewal in the mouse heart. Mice 2-3 months of age were injected every 12 hours with BrdU for 6 days prior to sacrifice. A second group of mice received one injection of 14C-thymidine and the animals were sacrificed from 1 to 90 days later. The BrdU method allowed us to measure the number of myocytes formed in 6 days. The 14C-thymidine strategy provided us with an evaluation of myocyte regeneration as a function of time. In assay 1, BrdU localization in myocytes was determined by immunolabeling and confocal microscopy. After 12 injections of BrdU, 3.1±0.4% or 100,000±13,000 left ventricular (LV) myocyte nuclei were labeled, indicating that a significant number of parenchymal cells was added to the LV in 6 days. Given that the number of LV myocytes is constant in the adult mouse heart, myocyte apoptosis was evaluated by tunnel assay, whereas myocyte necrosis was assessed by the incorporation of circulating vitronectin that reflects an irreversible loss of integrity of the plasma-membrane. Apoptosis comprised 186±55 myocyte nuclei/10^6 and cell necrosis was present in 280±103/10^6 cell profiles. Since apoptosis last at most 4 hours and necrosis 20 hours, there was a 2.9±0.4% drop out of myocytes in 6 days. In assay 2, LV myocytes were isolated, purified by differential centrifugation, and the DNA extracted for the measurement of the kinetics of ^{14}C inocropoation by Accelerator Mass Spectrometry. Our preliminary findings indicate that ^{14}C level in myocyte DNA increased progressively from 1 to 10 days. These observations suggest that a significant number of new myocytes was formed in 10 days. Thus, our initial observation indicate that myocyte turnover is high in the adult mouse heart. This work requires a more accurate evaluation of ^{14}C incorporation. This will be done during the next year of support.

c) One of our major objectives is to study the mechanisms that regulate the magnitude of myocyte turnover over organism's lifespan. During this funding period, we have employed cell cycle proteins, BrdU incorporation, phospho-H3 and aurora B kinase localization to evaluate cardiomyogenesis in the mouse heart. However, these determinants of myocyte growth are influenced by the length of the phases of the cell cycle imposing on us the development of a time-consuming and challenging protocol. For example, an extended S phase would increase the fraction of BrdU-positive cells, implying a higher turnover rate. To establish the number of left ventricular (LV) myocytes being formed in the young-adult, 2 months, and senescent, 30 months, mouse heart, a double “pulse” labeling protocol was used. Mice were injected with BrdU, and 4 hours later with EdU to measure the proportion of cells tagged by both thymidine analogs, and cells tagged by either BrdU or EdU. BrdU- and EdU-positive cells corresponded to cells in S phase, and cells positive for BrdU only reflected cells that entered G2-M. The BrdU-positive cells in G2-M defined the myocytes generated during the 4 hour interval between the delivery of BrdU and EdU, regardless of the length of the cell cycle and its two major phases, G1 and S. The nucleotide analogs employed could not discriminate whether DNA replication was associated with karyokinesis in the absence of cytokinesis, or ploidy formation; both would mimic myocyte growth resulting in an overestimation of cell turnover. These variables were measured in isolated cells: 89% of myocytes were binucleated, and mononucleated and multinucleated cells constituted 11% of myocytes in the young and old LV. Moreover, at the two ages, essentially all myocyte nuclei showed a 2n diploid DNA content, 98%; only a small fraction of nuclei was tetraploid, 2%, and octaploid nuclei were not detected. Based on these initial data, we established that in the 2 month-old heart 19.000±7,000 (0.6%), and 580,000±210,000 (18%), new myocytes were formed per day and per month, respectively. Corresponding values at 30 months were 50,000±18,000 (1.8%) and 1.5±0.5 million (54%). Therefore, LV myocytes were replaced entirely in 6 and 2 months in the young and old heart, respectively. Importantly, these rates of myocyte turnover were not influenced by changes in the number of nuclei per cell and polyploidy. Together, these results suggest that the adult mouse heart is a dynamic organ that renews its myocyte compartment several times during the course of life.

d) One of the major proposed objectives is to study the mechanisms that regulate the homeostasis of the aging heart. Specifically, the failure of the heart to adapt to aging and pathological loads may be dictated by alterations of the functional properties of the cardiac stem cell (CSC) niches and formation of abnormal sites of cardiomyogenesis. It is known that low oxygen tension favors hematopoietic stem cell quiescence while normoxia is required for their activation. We raised the possibility that CSC function may be similarly regulated and disruption of the balance between hypoxic and normoxic CSCs may occur with aging. We have documented that the fraction of hypoxic CSCs increased 1.5-fold in the mouse heart from 3 to 30 months of age. In young and old mice, hypoxic CSCs were quiescent and lineage negative while cycling and early committed cells were restricted to the normoxic pool. At 3 months, hypoxic CSCs carried significantly longer telomeres than normoxic CSCs. Telomere length did not decrease with age in hypoxic CSCs, while a 43% shortening occurred in normoxic CSCs, which showed higher levels of p16INK4a expression. The quiescent state of the larger compartment of CSCs with long telomeres in the senescent heart imposes a great growth demand on normoxic CSCs, which generate a myocyte progeny that is chronologically young, but phenotypically old.

e) An important question related to the effects of hypoxic on primitive cells of the heart, is whether hypoxic cardiac stem cells (CSCs) can be activated to generate mechanically efficient myocytes and rejuvenate the aged heart. For this purpose, stem cell factor (SCF) was delivered to the left ventricle of old mice. At 6 hours, hypoxic CSCs decreased 55% and normoxic CSCs increased 97%. At 2 days, SCF resulted in a 95% increase in the fraction of BrdU-labeled CSCs which led at 3 weeks to a 32% increase in CSC number. In SCF-treated mice, the fraction of BrdU-positive myocytes increased 114%; telomere length in these newly-formed myocytes was 75% longer than in BrdU-negative cells, indicating that regenerated myocytes derived from activation of CSCs with a younger cell phenotype. SCF decreased LV diastolic and systolic volumes and increased LV wall thickness. Diastolic and systolic wall stress decreased by 56% and 45% in treated mice, in which LV mass-to-chamber volume ratio increased 84% in diastole and 2.2-fold in systole. The expansion in LV mass was characterized by a 2.1-fold increase in the number of mononucleated myocytes. Conversely, binucleated myocytes decreased 12% in SCF-treated hearts. Thus, the compartment of quiescent, young CSCs can be stimulated in situ by SCF; this intervention repopulates the senescent heart with chronologically younger CSCs and myocytes, reversing the cardiac senescent phenotype in vivo.

f) A major objective of our proposal is to define the evolution of the aging process in large mammals. Initial evaluations by echocardiography and invasive hemodynamics in chronically instrumented conscious aging Beagle dogs have been implemented to determine whether the old canine heart shows alterations in diastolic and systolic performance, depressed exercise tolerance, and difficulties to increase its inotropic state. Our Data indicate that some relevant anatomical and physiological differences are present between young (3.1 years) and old (11.3 years) Beagle dogs. Heart weight increases with age and the expansion in cardiac mass is accompanied by left ventricular (LV) cavitary dilation and wall thinning. LV end-diastolic pressure (LVEDP), LV systolic pressure (LVSP), and +dP/dt are essentially maintained at baseline. However, a marked increase in diastolic and systolic stress at the LV base-mid-region and apex was detected. Additionally, ejection fraction (EF), -dP/dt, stroke volume and cardiac output were decreased. These age-dependent effects are aggravated further by exercise, pacing, and β adrenergic stimulation; these protocols show a significant reduction in exercise capacity, and inotropic reserve of old dogs. Thus, myocardial aging in Beagle dogs shows alterations in cardiac anatomy and function coupled with a decreased ability of the old heart to sustain a hemodynamic challenge. This study requires additional work that will be performed during the next year of support. Importantly, myocytes have been collected from young and old dogs, and analysis of atmospheric 14C will be performed to gain information related to the average age o f cardiomyocyte.

g) Aging is associated with alterations in the electrical properties of the heart resulting in an increased incidence of arrhythmic events and defective cardiac performance. The aim of this study was to determine whether the late sodium current (INaL), which presents slow inactivation kinetics, is upregulated in myocytes from old hearts, contributing to the electrical remodeling of the senescent myocardium. For this purpose, physiological determinations in vivo, in left ventricular (LV) preparations, and in isolated myocytes were conducted using young (3 years) and old (11 years) Beagle dogs. Hemodynamic and echocardiographic parameters indicated functional defects in old animals, which were aggravated following treadmill exercise test, pacing, and beta-adrenergic challenge. In the perfused LV myocardium, transmural ECG in old dogs presented prolonged QT interval with respect to young animals; the delayed electrical recovery was associated with a 13% increase in the effective refractory period and a 1.3-fold prolongation of monophasic action potentials (APs). By patch-clamp, isolated LV myocytes from old hearts presented longer AP duration (+31%), in comparison with young. In voltage-clamp mode, the late Na current, which is operative during the repolarization phase of the AP, was 2.3-fold larger in old myocytes with respect to young. Blockade of INaL in old cells with low doses of tetrodotoxin or ranolazine, shortened the AP by 45% and 32%, respectively. Additionally, stimulation frequency protocols revealed that both INaL amplitude and AP duration presented reverse rate dependency. Thus, these findings indicate that INaL represents the ionic basis for the prolonged APs in old cells. To test the possibility that INaL is a critical modulator of the electromechanical coupling in dog myocytes, Ca^{2+} cycling and contractility were evaluated in isolated cells. Blockade of INaL resulted in a 40% decrease in the amplitude of Ca2+ transients and cell shortening, and promoted faster Ca^{2+} decay and relaxation. In conclusion, upregulation of INaL prolongs the AP and provides inotropic support to the aging myocardium; re-activation properties of INaL may underlie the impaired rate-dependent cardiac reserve observed in old dogs.

f) One of the major objectives of the proposal is to demonstrate whether the aging cardiomyopathy is accompanied by dysregulation in the electromechanical properties of cardiomyocytes. Calcium release from the endoplasmic reticulum via inositol 1,4,5-triphosphate receptors (IP3Rs) regulate the growth and fate of human cardiac stem cells, but whether IP3Rs are operative in the derived myocyte progeny, and modulate Ca^{2+} homeostasis and electrical activity in this cell compartment is unknown. In this study, IP3R function was determined in left ventricular (LV) myocytes from normal human hearts declined for transplantation and integrated with assays in mouse cells. Transcripts for the three IP3R subtypes were identified in human LV myocytes by qRT-PCR and the expression of IP3R-2 was detected by Western blotting. In field-stimulated cells, IP3R activation via Gq-protein-coupled receptor agonists (endothelin-1, ATP) increased Ca^{2+} transient amplitude and contractility. Moreover, extra-systolic Ca^{2+} release and after-contractions were induced. These effects were prevented by IP3R blockade with xestopspongin-C. Similarly, myocytes obtained from mice infected in vivo with small hairpin RNA targeting IP3R-2, failed to respond to Gq-protein receptor agonists. By patch-clamp, IP3R stimulation in human LV myocytes resulted in a 1.5-fold increase in the time to 50% repolarization of the action potential and promoted the occurrence of early and delayed after-depolarizations. In voltage-clamp, IP3R activation in human and mouse cells elicited transient inward currents preceded by small elevations in intracellular Ca^{2+}. To test the possibility that IP3R- Ca2+ mobilization promoted the electrogenic extrusion of this cation causing electrical instability, the activity of the Na/Ca exchanger (NCX) was measured with nickel. Current-voltage relationships documented that IP3R agonists induced larger inward currents in the range of potentials corresponding to forward mode NCX. Alterations in the electromechanical characteristics of human cardiomyocytes following IP3R activation were coupled with a ~30% increased isometric twitch of LV trabeculae, and with a prolongation of epicardial monophasic action potentials and occurrence of arrhythmic events in perfused human LV myocardium. Thus, IP3R signaling provides inotropic reserve, which is hampered by the initiation of electrical disorders and contractile abnormalities. This study was recently accepted for publication in Circulation.

SPID 0065 Metabolite Profiling of cellulolytic microorganisms for biofuel production (C)

RR project # 70

Investigators:

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PROJECT DESCRIPTION: For established cellulose-based H_2-producing co-culture systems, modest H_2 production has been achieved in bioreactors with syntrophic microorganisms. However, the metabolic interactions between the organisms are poorly understood. The goal of this project is to characterize metabolic interactions between H_2-producing organisms in order to enable system optimization for improving H_2 production efficiency.

SPID 0066 Determination of the age of ruptured and unruptured intracranial aneurysms (C)

RR project # 73

Investigators:

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PROJECT DESCRIPTION:

Traditional methods used for dating cells are limited in the information they provide, or are not appropriate for human use. We developed a method for the retrospective birth dating of cells using bomb pulse carbon-14 (^{14}C) dating as a method for measuring the approximate age of specific populations of cells in the adult human brain and other tissues. This method is based on establishing the proportion of the isotope ^{14}C in genomic DNA.

After a cell has terminally differentiated it does not divide again. Since the last cell division represents the last time point when the cell synthesized DNA, its chromosomal DNA will reflect the age when the cell was born. Traditionally, the slow decay of ^{14}C relative to other carbon isotopes has given it a temporal resolution of many years, however due to nuclear tests in the late 1950s and early 1960s, the level of ^{14}C in the atmosphere doubled. This level has since dropped off in an exponential fashion, allowing one to resolve ^{14}C differences in the range of years. Because DNA has a ^{14}C content reflective of the time when it was synthesized, establishing the ^{14}C content of chromosomal DNA will enable us to retrospectively birth date cells, and thus establish cellular turnover.

Rather than dating DNA, the objective of this study is to establish a method to date ruptured and unruptured intracranial aneurysms in patients treated with aneurysm clipping and excision. Subarachnoid hemorrhage (SAH) due to ruptured intracranial aneurysm is a major cause of morbidity and mortality in patients with cerebrovascular incidents, especially in younger patients. It accounts for 10% of strokes and affects 10 of every 100,000 inhabitants in North-America and Europe. Despite the efforts to optimize treatment, aneurysmal SAH continues to have a case fatality of 25 to 50%. Due to the poor prognosis of aneurysm rupture, there has been great interest in methods to detect and treat intracranial aneurysms prior to rupture. This study tests the hypothesis that ruptured aneurysms rapidly increase in size shortly before the time of rupture, and that their actual size thus would not correspond to the size of the aneurysm at any time before rupture. We hypothesize that rupture of intracranial aneurysms occurs as a result of instability and growth. Since the aneurysm grew acutely before rupture, the ruptured aneurysm should consist mainly of younger or new aneurysmal tissue. This hypothesis is supported by the observation that there are a large number of small aneurysms found after SAH in epidemiological studies, suggesting that a high proportion of aneurysms arise rapidly and quickly progress to rupture. Contrary to patients with SAH due to small ruptured aneurysms, epidemiological data show there are older patients with larger incidental aneurysms. These cases might be explained through a selection effect where if aneurysm growth does not result in rupture, it could lead to an enlarged but stable aneurysm. Until further investigation it will remain controversial whether older patients who present with unruptured aneurysms that measure above average in diameter (i.e. more than 7-8 mm in diameter), have older aneurysms or whether these aneurysms are young and have progressed and formed rapidly.

The objective of this study is to establish a method to date ruptured and unruptured intracranial aneurysms in patients treated with aneurysm clipping and excision. However, these data are theoretical or observational and to date no study has analysed aneurysms from patients to determine the age of the aneurysm. We will test the hypothesis that unruptured aneurysms are older than ruptured aneurysms. The answer to our hypothesis would bear on the question of use of radiological screening of the population for incidental aneurysms. If aneurysms form rapidly and rupture or stabilize and never rupture, then screening may not be very useful. The results of the present study seek to determine whether this occurs.

We will determine age of ruptured and unruptured intracranial aneurysms using ^{14}C dating of aneurismal collagen, on the condition that the tissue is ultra-pure and not mixed with other tissues or cells. Hence, after isolation and purification of collagen from aneurysm walls excised from patients undergoing surgery, we will determine the age of the collagen using ^{14}C birth dating in order to understand aneurysm formation in patients with ruptured aneurysms compared to unruptured aneurysms.

Research Progress (through 3/31/2014):
A larger cohort of collagen from cerebral aneurysms was analyzed. Additionally collagen extraction from cadaveric cerebral arteries was attempted to compare collagen turnover in aneurysms to that in arteries. The paper was submitted in February 2014.

SPID 0067 Micro-Dosing to Determine the Pharmacokinetics of PAHs (C)

RR project # 74

Investigators:

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PROJECT DESCRIPTION:

PAHs (polycyclic aromatic hydrocarbons) are widespread pollutants that result from the burning of material such as cigarettes, diesel, coal, or gasoline. They are also found in foods such as smoked meat and cheese, or liquor aged in charred wood barrels. In animals, high levels of PAHs can cause cancer of the skin, breast and lung. In humans, PAHs are linked to cancer and other health problems such as asthma. It is unknown how fast PAHs are absorbed into the body when eaten, or how long they stay in the body. Even though PAHs are ubiquitous environmental pollutants of potential concern to human health, there is little or no information on the pharmacokinetics of PAHs in humans. With the utilization of Accelerator Mass Spectrometry (AMS), it is possible to safely measure ^{14}C-isotopically-labeled PAHs in human tissues following administration of micro-doses. The results from this study will be used to model the pharmacokinetics of polycyclic aromatic hydrocarbons (PAHs) in humans. This work has never been done before and the potential benefit for regulatory agencies is very significant. As of now, they have to rely on animal data. As PAHs are increasing in the environment, it is crucial that we have the best information possible to make decisions concerning safe exposure by inhalation or by diet.

****Research Progress (through 3/31/2014):**

^{14}C- Dibenzo[def,p]chrysene (DBC) from the National Cancer Institute was utilized in a pharmacokinetic study employing micro-dosing with analysis by Bio-AMS Research Resource at Lawrence Livermore National Laboratory. Nine volunteer's samples were processed with solid sample AMS, resulting in a pharmacokinetic profile that closely matched the computational model predictions generated by Pacific Northwest National Laboratory.

Table 1. Pharmacokinetic parameters following micro-dosing to humans of [14C]-DBC

Pharmacokinetic Parameters Of Human DBC Metabolism								
Volunteer	1.00	6.00	8.00	9.00	10.00	13.00	avg	sd
C Max (fg*mL^{-1})	124.12	118.23	30.45	41.63	74.98	23.27	68.78	44.33
T Max (hr)	1.50	1.50	1.50	4.00	2.00	3.00	2.25	1.04
AUC-t (fg*hr*mL^{-1})	1615.67	2850.26	589.16	432.98	1921.01	237.23	1274.39	1026.76
Total Kel (fg*h^{-1})	0.03	0.03	0.03	0.05	0.00	0.03	0.03	0.02
AUC-∞(fg*hr*mL^{-1})	1615.73	3690.42	699.07	603.78	6577.79	338.36	2254.19	2448.53
total T1/2 (h)	23.52	27.51	23.93	13.14	142.16	22.07	42.06	49.28
α Phase T1/2 (h)	6.82	11.71	4.84	1.69	5.81	3.82	5.78	3.40
α Phase Kel (fg*h^{-1})	0.10	0.06	0.14	0.41	0.12	0.18	0.17	0.12
α Phase AUC-t	462.11	859.14	179.74	30.71	236.90	74.84	307.24	309.92
β elim T1/2 (h)	23.56	66.61	28.28	10.16	88.63	30.64	41.31	29.83
β elim Kel (fg*h^{-1})	0.03	0.01	0.02	0.07	0.01	0.02	0.03	0.02

Currently the pharmacodynamics of DBC metabolism are being investigated with the moving wire interface between UPLC and liquid sample AMS. Preliminarily, we have identified a UPLC method to separate the ^{14}C-DBC metabolites in plasma and urine extract prior to detection with AMS. Progress is being made to generate data of human DBC metabolism over time and across volunteers.

SPID 0068 Transport of ^{14}C-TCC Across The Placental Barrier (C)

RR project # 75

Investigators:

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PROJECT DESCRIPTION:
It has been demonstrated that a variety of chemicals are not removed after wastewater treatment, which results in their release back into the water supply and the environment. These contaminating chemicals may act as endocrine disrupting compounds (EDC), which can not only affect the function of the endocrine system, but also adversely affect progeny. Studies determining the effects of EDC during periods of development are lacking, including quantitative measures of accumulation after exposure. Therefore, within this project we are investigating the transfer of an EDC, triclocarban (TCC), from mother to offspring both *in utero* and through lactation using AMS. These data can be correlated with the developmental outcomes observed after exposure.

**Research Progress (through 3/31/2014):
In the first two years of this project, we successfully developed a cell culture model of the human placental barrier and determined the transfer of low environmentally relevant concentrations of TCC. We observed that <10% of the doses tested (10, 1 and 0.1nM) were capable of transferring into the fetal compartment over an 8 hour time course. In addition to the *in vitro* studies, we have developed and validated drinking bottles for the *in vivo* TCC exposure studies performed in the third year. This last year (year 3) we have completed and analyzed the *in utero* and lactation exposure studies for 100nM TCC. We found detectable levels of TCC in offspring from TCC exposed mothers. For gestational (*in utero*) exposure, 0.005 %ID/g (ID= ingested dose) +/- 0.0009 (SEM) was detected in offspring. A much higher concentration of 0.015** %ID/g +/- 0.002 was observed in offspring from mothers exposed during lactation (**p<0.01). We also observed significant changes in offspring weight for both gestation (*p<0.05) and lactation groups (***p<0.001), which indicated that exposure of offspring to TCC may be related to increases in body weight. We are currently investigating whether this increase in offspring weight remains significant when adulthood is reached.

SPID 0069 Multi-Scale Toxicology Initiative (C)

RR project # 76

Investigators:

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PROJECT DESCRIPTION:

The purpose of this project is to develop a suite of new capabilities for assessing the safety of materials designed for use in humans or to which humans will be exposed. Assessing potential safety/toxicity of nanomaterials and cardiotoxic drugs will be the focusing problems for this effort. The goals are to combine existing measurement capabilities into a unique multiscale toxicology assessment pipeline, to integrate data from different end-point studies into a comprehensive model that links the *in vitro* cellular effects to the *in vivo* whole animal response and most importantly to develop a suite of new high throughput technologies that, when combined, will add new insights into the potential toxicities of materials developed for use in humans. Such insights should reduce the time it takes to complete safety assessments and thus reduce the time it takes to make new products available to the public. It should also improve the accuracy of predicting human toxicity. Importantly, the end products of this research will be of similar value for assessing the safety of chemical agents used in commerce as well as those found in the environment. Nanoparticles are a class of materials that is receiving increasing interest for a variety of applications ranging from electronics, catalysis, photovoltaic materials to drug delivery devices. Research on the application of nanomaterials is intensifying and over 200 potential product uses are being investigated. One general area of use that has received much attention is in health care. Certain properties of nanomaterials make them ideal candidates for use in drug delivery, medical diagnostics & imaging, implantable devices, biomaterials or as pharmaceuticals themselves. While much emphasis has been invested in exploring applications of nanomaterials, relatively little effort has been directed towards understanding the health risks associated with their use. Use of nanomaterials in pharmaceuticals and medical devices will require safety evaluations as part of the development process prior to FDA approval. AMny drugs have side-effects that can cause cardiotoxicity, which can limit the dose that can be administered. By developing tools and methods to detect indications cardiotoxcity before deleterious effects occur, specific dosing regimes can be developed to avoid the adverse side effects from these drugs and increase the safety margin.

****Research Progress (through 3/31/2014):**

Doxorubicin concentration in cardiac tissue

Doxorubicin (Dox), a common chemotherapeutic, has been shown to cause cardiotoxicity even at very low doses. However, the exact mechanism of its cardiotoxic effects is not well understood. Additionally, its metabolites have been shown to be more toxic than Dox itself. Therefore, a better understanding of time specific cardiac concentrations of Dox and its metabolites and their correlation to cardiac function is needed. In this study, accelerator mass spectrometry was used to measure the concentrations of doxorubicin and two metabolites: doxorubinol and doxorubicinolone over 21 days in different regions of the heart after intravenous dosing (2mg/kg) in rats. Detectable levels of Dox and both metabolites were observed over 21 days in all regions of the heart with doxorubicinol containing the highest concentration (~0.2µg/g) in cardiac tissue. In a parallel study, the Langendorff heart model was used to investigate cardiac function after Dox exposure. Slight but insignificant reductions in contractility and relaxation speeds were observed for the Dox group. Significant reductions were found for heart rate and perfusion flow rate; no changes were observed for ECG intervals and morphology. The current effort of this project is to assess main organs of Dox metabolism for correlation with cardiac concentrations observed over 21 days.

SPID 0070 Novel Agents for Gram-Negative Biodefense Pathogens (C)

RR project # 77

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PROJECT DESCRIPTION:

This project is to discover and develop a novel broad-spectrum antimicrobial compound active against the priority bacterial pathogens in the Strategy and Implementation Plan for Chemical, Biological, Radiological and Nuclear Threats. The top priority threats addressed include *F. tularensis* and *Y. pestis.* The aim of the research is to provide a single solution to multiple threats, complementing older antibacterials that are already known to be vulnerable to resistance mechanisms. The overall scope of the work ensures that multiple compounds will advance to optimization, allowing for multiple opportunities for success. The goal in optimization is to advance one to three promising compounds into preclinical evaluation. The net result of this project will be an antimicrobial agent with unprecedented spectrum and safety.

To assess the efficacy of these leads in vivo testing need to be done, including determining the pharmacokinetics (PK) and metabolism of the drug leads . Very low concentrations of drug and metabolites can be readily assessed by the application of Accelerator Mass Spectrometry to metabolite analysis, PK assessment, protein binding and tissue distribution studies. AMS is used to achieve the sensitivity needed for low dose studies of both animal and human samples or tissue distribution studies. Whereas current methods of decay counting usually require high doses with high-specific-activity compounds, AMS is exquisitely sensitive and selective for ^{14}C analysis. We can quantify with high precision the distribution of lead compounds (and their metabolites) through the body at 100 – 1000-fold lower concentrations than those likely to be required for therapeutic efficacy which is not achievable using decay counting methods

SPID 0071 New broad spectrum antimicrobial for multi-drug resistant biowarfare pathogens (C)

RR project # 78

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PROJECT DESCRIPTION:

This project is to discover and develop a novel broad-spectrum antimicrobial compound active against the priority bacterial pathogens in the Strategy and Implementation Plan for Chemical, Biological, Radiological and Nuclear Threats. The top priority threats addressed include *F. tularensis* and *Y. pestis.* The aim of the research is to provide a single solution to multiple threats, complementing older antibacterials that are already known to be vulnerable to resistance mechanisms. The overall scope of the work ensures that multiple compounds will advance to optimization, allowing for multiple opportunities for success. The goal in optimization is to advance one to three promising compounds into preclinical evaluation. The net result of this project will be an antimicrobial agent with unprecedented spectrum and safety.

To assess the efficacy of these leads in vivo testing need to be done, including determining the pharmacokinetics (PK) and metabolism of the drug leads . Very low concentrations of drug and metabolites can be readily assessed by the application of Accelerator Mass Spectrometry to metabolite analysis, PK assessment, protein binding and tissue distribution studies. AMS is used to achieve the sensitivity needed for low dose studies of both animal and human samples or tissue distribution studies. Whereas current methods of decay counting usually require high doses with high-specific-activity compounds, AMS is exquisitely sensitive and selective for ^{14}C analysis. We can quantify with high precision the distribution of lead compounds (and their metabolites) through the body at 100 – 1000-fold lower concentrations than those likely to be required for therapeutic efficacy which is not achievable using decay counting methods

SPID 0073 Feasibility of nanoparticle-mediated Paclitaxel delivery for Phase 0 Clinical Studies (C)

RR project # 66

Investigators:

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PROJECT DESCRIPTION: Paclitaxel (Taxol) is a standard and effective chemotherapeutic for many cancer types, including breast cancer, ovarian cancer, small cell lung cancer and non-small cell lung cancer. Since paclitaxel (PTX) has very limited solubility in water, the formulation of this drug requires Cremophor EL which causes significant side effects, such as allergic reactions. Consequently, patients receiving PTX require premedication with histamine blockers and steroids. We proposed to use a novel water-soluble nanoparticle-based formulation to enable better drug delivery of PTX. This proprietary formulation has been demonstrated to be safer and more effective in preclinical studies compared to currently available formulations. For example, some tumor-bearing mice exposed to PTX-nanoparticles were cured of cancer, which was not observed for the Cremophor formulation. The goal of this study is to advance the use of PTX-nanoparticles into Phase 0 clinical studies by using 14C-paclitaxel to label the nanoparticles followed by absorption, distribution, metabolism and excretion (ADME) studies in nude mice with human tumor xenografts. The particles will be synthesized according to established protocols which includes addition of the 14C-paclitaxel in the final self-assemble step. Mice will be dosed with PTX-nanoparticles of sufficient specific activity to allow tracing by liquid scintillation counting (LSC). The LSC experiments will allow calculation of the specific activity needed for the studies to be repeated using AMS-based detection of the 14C-paclitaxel. AMS is needed as part of the project in order to predict dose formulations and develop methods for use in clinical studies (to be proposed later). It is anticipated that LSC has sufficient sensitivity for human phase 0 studies, but this needs to be established empirically with the proposed animal studies. If successful, the feasibility study data will be submitted to FDA for an exploratory IND application in order to determine the pharmacokinetics of PTX-nanoparticles in humans in order to justify subsequent Phase 1 studies.

****Research Progress (through 3/31/2014):**

Background: A multifunctional telodendrimer-based micelle system was characterized for delivery of imaging and chemotherapy agents to mouse tumor xenografts. Previous optical imaging studies demonstrated qualitatively that these classes of nanoparticles, called nanomicelles, preferentially accumulate at tumor sites in mice. The research reported herein describes the pharmacokinetics and biodistribution profiling of nanomicelles loaded with a cargo of paclitaxel in rats.

Methods: The nanomicelles were loaded with ^{14}C-paclitaxel, which allowed measurement of pharmacokinetics and biodistribution in cannulated rats using AMS.

Results: The pharmacokinetics of the nanomicelle formulation features much slower serum half lives in rats compared to paclitaxel formulated in Chremophor/EL, a formulation that is used in the clinic. However, uncrosslinked nanomicelles containing paclitaxel were more toxic than the Chremophor/EL or a crosslinled nanomicelle formultation, as evidenced by death from acute toxicity of several of the animals from the nanomicelle arm versus no deaths in the other treatment arms.

Conclusion: Overall, the results indicate that nanomicelle-formulated paclitaxel is a potentially superior formulation compared with Taxol in terms of water solubility, pharmacokinetics, and tumor accumulation, and may be clinically useful for both tumor imaging and improved chemotherapy applications upon optimization of the nanoparticle formulation.

SPID 0074 Quantitative analysis of cell turnover: a strategy for personalising leukaemia treatment (C)

RR project # 81

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PROJECT DESCRIPTION:

B-Cell chronic lymphocytic leukaemia (CLL) is a heterogeneous disease with variable clinical course. A fundamental drawback of CLL management is the lack of sensitive early indicators of disease progression to guide and monitor intervention strategies in individual patients. Recent studies have identified a proliferative compartment in every patient, indicating that increased cell birth rate, as well as resistance to apoptosis, contributes towards rising lymphocyte count.

This project aims to examine the potential clinical value of a newly-designed technique to measure CLL cell birth and death rates *in vivo*. In this approach, [^{14}C]-labelled aspartic acid is incorporated into the DNA of actively proliferating CLL cells. The degree of ^{14}C incorporation and subsequent loss of labelled cells from the circulation of an individual may identify prospectively patients destined to develop progressive disease. The strategy, which utilizes accelerator mass spectrometry (AMS), offers significant advantages over labelling methods involving deuterated water, due to vastly superior sensitivity, which permits kinetic profiling of smaller numbers of cells. The technique may also help assess sensitivity to different forms of chemotherapy by monitoring induction of apoptosis and/or inhibition of proliferation caused by chemotherapeutic agents, permitting the rational introduction of personalised therapy.

****Research Progress (through 3/31/2014):**

To ascertain whether [^{14}C]-aspartic acid is incorporated into cellular DNA of human leukaemia cells and define the kinetics of this process, MEC-1 cells were exposed to a short pulse of physiologically achievable concentrations, the medium was then replaced and incubation continued. After harvesting, DNA was extracted, quantified and subject to AMS analysis. All DNA samples from cells treated with [^{14}C]-aspartic acid contained significantly higher levels of ^{14}C compared to untreated controls, with maximal incorporation at ~4 h, followed by a gradual decline over 4 days. Elevated ^{14}C levels were still evident in DNA after 96 h, illustrating the potential for longer term tracking. Using patient derived CLL cells, [^{14}C]-aspartic acid was also incorporated into DNA, as demonstrated by a small significant increase above background, but the extent of maximal incorporation was far below that detected in the MEC-1 cells, and the levels failed to decline over the analysis period, which probably reflects the lower proliferative capacity of primary cells *in vitro*. Importantly, these studies have helped to define the number of cells and amount of DNA required for successful AMS analysis.

To demonstrate that the increased ^{14}C detected in DNA is due to covalent incorporation into the DNA molecule MEC-1 cells were treated with [^{14}C]-aspartic acid, using the optimised protocol outlined above. Cells were harvested at 12 h for DNA extraction and control incubations were conducted in parallel. DNA was digested using established protocols and fractionated using a HPLC chromatographic method developed for the detection and separation of DNA nucleosides. HPLC fractions were collected over 24 min, concentrated to dryness and analysed by AMS. Reconstruction of the HPLC-AMS chromatograms revealed four distinct peaks in the digested DNA isolated from [^{14}C]-aspartic acid-treated cells, which correspond in retention time to the individual deoxyribonucleosides, with preferential incorporation into pyrimidines. Importantly, there were no peaks of ^{14}C in the control DNA digest; all samples contained the expected background level of radiocarbon.

To provide proof of principle that the approach under development can be used to monitor the turnover of specific cellular subpopulations *in vivo* we are conducting *in vitro* experiments before translating to mouse xenografts. To this end we have identified a leukaemia cell line (U2932 cells) that contains at least two distinct populations that can be characterised by FACS analysis. These cells consist of two separate stable clones which can be distinguished based on CD20 and CD38 expression. [^{14}C]-Aspartic acid incorporation studies will be performed with U2932 cells and individual populations isolated by FACS sorting. DNA extracted from these cells will be analysed by AMS. Importantly, we have confirmed that the CD20/CD38 antibodies for labelling U2932 cells are human specific, with no detectable crossover with mouse lymphocytes. Therefore, these antibodies can be used for selective isolation of human cells by flow cytometry, which is essential for the xenograft studies.

SPID 0075 Determination of the age of arteriovenous malformations (C)

RR project # 83

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PROJECT DESCRIPTION:

The objective of this study is to establish a method to date arteriovenous malformations (AVMs). Brain AVMs (BAVMs) are complexes of tortuous, tangled vessels with fistulous connections between arteries to veins without a true capillary bed. AVMs are much larger than aneurysms so it is possible to date DNA of vascular cells comprising them and the structural collagen or elastin.

BAVMs are thought congenital in etiology, though there are a handful of cases reports documenting de novo post-natal BAVM formation. Of this small number of cases of de novo formation, patients more often had an additional pathological vascular anomaly suggesting a possible response-to-injury mechanism underlying AVM development. There is, however, limited data available regarding specific inciting events that may lead to a BAVM when acquired, or causes of incomplete penetrance when familial (the vast majority (>95%) are not familial). AVMs may therefore consist of cells and collagen of various ages. The age or turnover of normal vascular tissue is also unknown.

AVMs are retrieved during surgery at UCSF and enter the tissue bank. The tissue would be disposed under usual circumstances. 20 AVM samples will be analyzed initially. Collagen and DNA will be extracted at UCSF or in collaboration with Nima Etminan and Rita Dreier in Germany.

Research Progress (through 3/31/2014):
An initial set of samples was processed in 2013 but collagen yield was unexpectedly low. A procedure for isolating the structural protein elastin is now being used.

SPID 0076 Trophic Transfer of ^{14}C-Labeled Carbon Nanotubes (C)

RR project # 80

Investigators:

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Elijah Petersen Co-PI NIST

Monika Mortimer, Assistant Specialist III (postdoctoral level), UC Santa Barbara, Bren School

John H. Priester, Associate Specialist (researcher), UC Santa Barbara, Bren School

Junyeol Kim, grad student, UC Santa Barbara, Bren School

PROJECT DESCRIPTION:

Engineered carbon-based nanomaterials are increasingly manufactured and used in various applications including clinical, thereby enhancing their distribution to various environments *in vivo* and *ex vivo*. Little is known regarding biological fates of carbon-based nanomaterials, including toxicity and trophic transfer with implications for biomagnification. This project is newly assessing the potential for trophic transfer of bacterially-bioaccumulated multi-walled carbon nanotubes (MWCNTs) to a protozoan predator, and will quantify the process. Effects on growth of each, prey and predator, are in quantification, as is the potential for bacterial mineralization of MWCNTs by a metabolically-versatile, including hydrocarbon-metabolizing, bacterial strain. While MWCNTs are known to be antibacterial in monocultures and mixed cultures, including biofilms, little is known about their recalcitrance during bacterial growth or their trophic transfer, once MWCNTs are associated with bacterial membranes or enter the cells. Enzymatic degradation has been previously demonstrated by others for MWCNTs in abiotic conditions, but bacterial mineralization has not. Impaired predation upon bacterial prey was shown by others, for protozoans that had first taken up CNTs, but the system studied here—where bacteria are pre-loaded with MWCNTs then fed as prey to protozoan predators—represents a realistic, yet untested, scenario for MWCNT trophic transfer initiated at the base of microbial food webs.

Unlabeled and ^{14}C-labeled MWCNTs have been synthesized by Dr. Elijah Petersen (NIST), using previously demonstrated methane chemical vapor deposition methods. The quantity of incorporated radiolabel is being determined by scintillation counting, and also by accelerator mass spectrometry (AMS) as per Dr. Bruce Buchholz (LLNL). Bacteria are cultured, initially with unlabeled (cold) MWCNTs, to establish the dose-response relationship during growth and to assess MWCNT association with cells via fluorescence and electron microscopy. Using effective MWCNT dose and cultivation conditions that result in association (i.e. bioaccumulation), bacteria are then cultured with ^{14}C-labeled MWCNTs and harvested at late exponential phase, then separated by centrifugation from unassociated MWCNTs in the growth media. During growth, biomineralization (i.e. conversion to $^{14}C\text{-}CO_2$) of ^{14}C is determined by base (NaOH)-trapping gas within a gas-tight Nephelo flask, as before. Cells are resuspended in starvation media used for trophic transfer studies with *Tetrahymena thermophila*, a protozoan predator. Protozoan population growth is monitored by direct counts as before. The major fractions in the trophic transfer experiment are separated then assessed for label incorporation, using AMS and also oxidation/ scintillation counting: unmetabolized bacteria (separated by filtration), protozoa (washed, following recovery by filtration), fecal pellets (in media, by centrifugation of filtrate). Whole (unfractionated) culture and fractions (as above) are quantified for radiolabel abundance, and compared to the amount of administered label to ensure mass balance. Radiolabel abundance is assessed in two to three ways: i) scintillation counting of oxidized samples, ii) graphite conversion and AMS, and iii) scintillation counting of trapped radiolabeled off-gas in MWCNT mineralization experiments.

In addition to core scientific discoveries in MWCNT biomineralization and trophic transfer, ^{14}C-MWCNTs and use approaches are to be made newly available to the collaborators, and AMS is to be demonstrated for this novel application. AMS is critical for sensitively assessing ^{14}C label accumulation on bacteria, and in the predators and cellular compartments. The activity levels are expected to be ample for AMS, including for very low MWCNT concentrations that need to be studied but for which scintillation counting would be inadequate and unable to resolve the realistically-low signals.

****Research Progress (through 3/31/2014):**
Doctoral student training and generation of bacterial growth curves in growth media.
Literature review on MWCNTs' effects on bacteria and protozoa.
Recruitment of a postdoctoral scholar for this project.

SPID 0077 Quantification of 14C by Optical Spectrometry (C)

RR project # 82

Investigators:

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PROJECT DESCRIPTION:

Quantification of ^{14}C via optical spectroscopy offers an approach that eliminates many of the shortcomings of an accelerator-based system, such as high cost and complex sample preparation procedures. This technique can exploit the selectivity of the ro-vibrational transitions in the infrared spectral region and their isotopic shifts. The sensitive infrared-based approach utilizes an intra-cavity laser technique to increase the optical path-length called Cavity Ring Down Spectroscopy (CDRS).

We will develop a table-top sized spectroscopic method to quantify ^{14}C in milligram sized biological samples at levels down to the isotopic natural abundance (^{14}C:C ratio of 1:10^{12}) using a technique called Cavity Ring Down Spectroscopy with excitation lines in the mid-infrared. We will utilize specific wavelengths in the midIR to optically measure the quantities of ^{14}C. CRDS is a method that has been used to quantify trace gases, including CO_2. This has been proven for ^{13}C and commercial instruments are in use for total CO_2 and delta-^{13}C measurements. Specific absorption lines exist that can be used to quantify $^{12}CO_2$, $^{13}CO_2$, and $^{14}CO_2$ in the same sample creating the opportunity for dual isotope labeling studies. Methods developed and in routine use for AMS analysis will be used to convert biological samples to CO_2 for analysis. We propose here to build and prove the concept that $^{14}CO_2$ can be quantified at levels useful for high sensitivity ^{14}C quantitation from biological samples.

**Research Progress (through 3/31/2014):

We have constructed a prototype instrument and used it to demonstrate the feasibility of our approach. We saw clear signal from a sample of CO_2 with a ^{14}C/C concentration that was approximately 100 times the contemporary atmospheric levels. Our prototype type lack several key design features that will be incorporated into our next generation instrument. That system should be sensitive enough to measure CO_2 with $^{14}C/C$ concentrations of $5x10^{-13}$, or ~0.5 Modern.

SPID 0078 Determination of the age of neuronal histones in adult human brain (C)

RR project # 84

Investigators:

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PROJECT DESCRIPTION:

Since their discovery in the late 1960's, the existence of histone variants has suggested an alternative mechanism for introducing small sequence variations into the eukaryotic epigenome, phenomena that are now known to govern fundamental aspects of chromatin structural organization, nucleosomal dynamics and transcription. Recent discoveries that mutations in specific histone variants and their associated chaperones contribute to human disease suggest an essential function for histone variant regulation during critical periods of cellular development. For decades, nucleosomal histones have been considered highly stable proteins with predicted half-lives of several months to years in non-dividing cells. Recent analysis of H3 and H4 dynamics in living cells has challenged this notion by showing that newly synthesized variant histones are rapidly incorporated at promoters, enhancers, regulatory elements and coding regions of highly transcribed genes. These findings raise the intriguing possibility that subpopulations of nucleosomal histones at "active," or dynamic, chromatin are rapidly turned over to regulate patterns of gene expression associated with plasticity in post-replicative neurons.

The variant histone H3.3 is specifically enriched at transcriptionally active genes and within gene promoters, at certain heterochromatic loci and at regulatory elements in mammalian cells. Unlike canonical H3 proteins, which require mitosis for active nucleosomal deposition, H3.3 is efficiently transcribed and incorporated in chromatin in a DNA replication-independent manner in post-mitotic neurons, suggesting a potential role for H3.3 in activity-dependent nucleosomal reorganization. Both in primary culture and in adult hippocampal neurons, we have used a combination of proteomic, genomic and functional analyses to demonstrate that H3.3 expression, turnover kinetics and genomic deposition are tightly regulated by alterations in neuronal activity. Specifically, our data indicate that sub-populations of H3.3 are rapidly turned over in a proteasomal-dependent manner in neuronal chromatin following periods of cellular activity, and that these dynamics are essential to transcriptional events necessary for establishing and maintaining various aspects of synaptic development and physiological plasticity in embryonic and adult neurons, respectively. Furthermore, direct manipulations of H3.3 nucleosomal dynamics in adult hippocampus reveal an essential role for histone variant exchange during periods of learning and memory formation.

Although it is clear that histone variant dynamics play essential roles in the maintenance of basal neuronal function in the rodent brain, it remains unclear as to whether such dynamics occur or have functional outcomes in the adult human CNS. To test this, we plan to make use of a recently developed technique known as bomb pulse carbon-14 (^{14}C) dating to measure the approximate age of specific populations of histones from human brain. Histones will obtained using standard histone purification procedures followed by reverse phase liquid chromatography (RP-HPLC) from adult human brain, and then subjected to bomb pulse ^{14}C dating to determine the turnover kinetics of H3.3 in the adult human CNS.

Research Progress (through 3/31/2014):

A series of control mouse samples are being processed in early 2014. Anticipate analysis of human samples in March 2014. A paper is in preparation.

SPID 0079 The Role of Altered Metabolism in Clear Cell Renal Cell Carcinoma and Chronic Kidney Disease (C)

RR project # 85

Investigators:

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PROJECT DESCRIPTION: Metabolic alterations have been observed in clear cell renal cell carcinomas (RCC), but the role of these changes in the progression and pathology of disease remain unclear. Chronic kidney disease (CKD) is a risk factor for renal cell carcinoma through unknown mechanisms, possibly including acidosis, dialysis, uremic toxins, impaired immunity, inflammation, and hypertension. Our research is focused on tumor requirements for amino acids not required by normal cells. To perform its role of regulating blood pH, the kidney has a tremendous requirement for glutamine. This amino acid is metabolized to glutamate with the concomitant release of ammonium ion which removes acid from body stores by trapping this ion in the urine. Glutamine and its downstream metabolites have been shown to inhibit apoptosis and oxidative stress. We hypothesize that the acidic and glutamine rich environment caused by CKD causes excess glutamine metabolism which supports renal cancer cell survival through inhibition of apoptosis and oxidative stress. We propose to perform pathway analysis using ^{14}C-glutamine to identify and quantify differences in glutamine metabolism between normal kidney and RCC cells and between RCC xenograft in the control rats and CKD model rats. These experiments will identify specific metabolic alterations in RCC cells, with important implications for treatment and prevention of these cancers.

Research Progress (through 3/31/2014): The first step of this project is to validate serum glutamine level of the CKD model rats. We have collected the first set of serum to analyze for glutamine (and creatinine at the UCD clinical lab), and in the meantime further in vivo experiments are underway in the 5/6 nephrectomy CKD rat model. In vitro preliminary experiments confirmed that glutamine depletion inhibits RCC cell viability and make the cells sensitive to ROS stress. We have been establishing methods to measure glutamine and its downstream metabolites in the media and will measure levels of those metabolites in rat serum.

SPID 0080 AMS to Determine [^{14}C]Tetramethylenedisulfotetramine Binding to GABA$_A$ Receptor (C)

RR project # 86

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Bruce Buchholz, LLNL

PROJECT DESCRIPTION:

Tetramethylenedisulfotetramine (TETS) is one of the simplest, easily synthesized and readily-available convulsant rodenticides and potential chemical terrorism agents with hundreds of human poisonings from accidental exposure or intentional use. It is one of the most feared candidate chemical terrorism agents.□TETS is a noncompetitive antagonist of the γ-aminobutyric acid type A receptor (GABA$_A$R) based on multiple physiological criteria and GABA$_A$R assays with two trioxabicyclooctane radioligands, *t*-butylbicyclophosphorothionate ([^{32}S]TBPS) and 4′- ethynyl-4-n-[^{3}H]propylbicycloorthobenzoate ([^{3}H]EBOB) with IC$_{50}$ values in rat brain membranes of 200 and 400 nM, respectively. TETS is a competitive inhibitor of [^{3}H]EBOB binding with a potency in the native rat GABA$_A$R consistent with its toxicity in mice. However, it does not inhibit all native GABA$_A$R preparations equally and does not inhibit the human recombinant β3 homopentamer GABA$_A$R. These deductions are based on the use of other radioligands to assay the activity of TETS. A direct answer on the TETS binding site requires the use of TETS itself as the radioligand. TETS was recently radiolabeled with ^{14}carbon for the present toxicological studies. An earlier attempt at tritium exchange with TETS and a catalyst did not introduce the desired label but instead resulted in decomposing the TETS. Radioligand binding studies for neuroreceptors and most toxicant targets normally require very high specific activities. [^{14}C]TETS used here has a specific activity of 14 mCi/ mmol which is only about 0.1-0.4% the level normally used for radioligand binding assays. The required quantitation can only be achieved for [^{14}C]TETS by greatly enhanced sensitivity resulting from accelerator mass spectrometry (AMS) which to our knowledge has never been used before in neuroreceptor radioligand binding assays. This study characterizes the [^{14}C]TETS and [^{3}H]EBOB binding sites in rat brain membrane GABA$_A$R. TETS and EBOB are compared as an unknown versus a standard cage convulsant radioligand which sometimes share a common binding site. Rat brain is used because TETS is primarily a rodenticide. The same experiments are run with [^{14}C]TETS and [^{3}H]EBOB so that only the radioligand and analytical method are varied to best evaluate the utility of the new radioligand and the AMS analysis technique. The preliminary studies reveal specific saturable binding of [^{14}C]TETS to rat brain membranes from which the GABA has been removed. Comparisons are made between

[^{14}C]TETS and [^{3}H]EBOB in competition with GABA$_A$R modulators and candidate antidotes. Inhibitors of [^{14}C]TETS binding will be tested in other laboratories as alleviatory agents for TETS toxicity. The receptor studies are made at UC Berkeley and the AMS analyses at LLNL. AMS is the only technique with adequate sensitivity to solve this problem.

Research Progress (through 3/31/2014):
Neuroreceptor binding studies of [^{14}C]TETS with rat brain membranes were compared with the standard GABA$_A$R radioligand 4'-ethynyl-4-*n*-[^{3}H]propylbicycloorthobenzoate ([^{3}H]EBOB) (46 Ci/ mmol). Ten noncompetitive antagonists of widely diverse chemotypes inhibited [^{14}C]TETS and [^{3}H]EBOB binding to a similar extent (r^2=0.90), but there were two exceptions, the insecticides fipronil and α-endosulfan**.** A paper is nearly complete.

Publications

1. Alkass, K., Saitoh, H., Buchholz, B.A., Bernard, S., Holmlund, G., Senn, D.R., Spalding, K.L., Druid, H. (2013) "Analysis of radiocarbon, stable isotopes and DNA in teeth to facilitate identification of unknown decedents", ***PLoS ONE.*** 8 e69597. **(SPID 0002, 0025)**

2. Cimino G.D., Pan C.X., Henderson P.T., (2013) "Personalized medicine for targeted and platinum-based chemotherapy of lung and bladder cancer." ***Bioanalysis***. 5(3):369-91. **(SPID 0048)**

3. Etminan, N., Dreier, R., Buchholz, B.A., Bruckner, P., Steiger, H.-J., Hänggi, D., MacDonald, R.L., (2013) "Exploring the age of intracranial aneurysms using carbon birth dating: preliminary result. ***Stroke*** 44(3) 799-802. **(SPID 0002, 0066)**

4. Etminan, N., Buchholz, B.A., Dreier, R., Bruckner, P., Torner, J.C., Steiger, H.-J., Hänggi, D., Macdonald, R.L., "Cerebral aneurysms: Formation, progression and developmental chronology". ***Translational Stroke Research*** 294 (2013) *in press*. **(SPID 0002, 0066)**

5. Falso M.J.S., Buchholz B.A., (2013) "Bomb pulse biology". ***Nucl. Instr. and Meth. B,*** 294, 666-670. **(SPID 0002)**

6. Ognibene, T.J., and Salazar, G. A., (2013). "Installation of hybrid ion source on the 1-MV LLNL BioAMS spectrometer." ***Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*** 294(0): 311-314. **(SPID 0001)**

7. Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H., Boström, E., Westerlund, I., Buchholz, B.A., Possnert, G., Mash, D., Druid, H. Frisén, J., "Dynamics of hippocampal neurogenesis in adult humans". ***Cell,*** 153 (2013) 1219-1227. **(SPID 0002, 0025)**

8. Salazar, G., and Ognibene, T. (2013) "Design of a secondary ionization target for direct production of a C^- beam from CO_2 pulses for online AMS." ***Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*** 294(0): 300-306. **(SPID 0001)**

9. Stewart B.J., Navid A., Kulp K.S., Knaack J.L., Bench G., (2013) "D-Lactate production as a function of glucose metabolism in Saccharomyces cerevisiae". ***Yeast***. 30(2):81-91. **(SPID 0002)**

10. Stewart, D.N., Lango, J., Nambiar, K.P., Falso, M.J.S., FitzGerald, P.G., Hammock, B.D., Buchholz, B.A., (2013) "Carbon turnover in water-soluble protein of the adult human lens". ***Molecular Vision*** 19:463-475. **(SPID 0002, 0055)**

11. Thomas, A.T., Stewart, B.J., Ognibene, T.J., Turteltaub, K.W., and Bench, G. (2013). "Directly coupled HPLC-AMS measurement of chemically-modified protein and peptides", ***Analytical Chemistry***, 85 (7): 3644-3650. **(SPID 0001, 0002)**

Investigator Support

Non-Federal & Federal-Non PHS

Investigator Name: Ted Ognibene
Title: PLS-Revolutionizing AMS Throughput
Organization: DOE/ISCP
Grant/Contract Number: 39590
Funds: $350,000/ yr
Subproject: SPID 0001

Investigator Name: Gabriela Loots
Title: Optimizing Drug Efficacy through Pharmacogenomics-Driven Personalized Therapy
Organization: LLNL/DOE
Grant/Contract Number: LDRD-ER- 13
Funds: $550,000
Subproject: SPID0003

Investigator Name: Kit Lam
Title: *Multifunctional nanoporphyrin against bladder cancer*
Organization: DoD PRMRP
Grant/Contract Number: PR121626
Funds: $ 400,000
Subproject: SPID 0073

Investigator Name: Pan/Henderson, Co-PIs
Title: Use of AMS method to analyze DNA damage/repair kinetics of ABT-888 + carboplatin in tumor cells.
Organization: Abbott Laboratories
Grant/Contract Number: UC Davis Project #201121855
Funds: $79,122
Subproject: SPID 0073

Investigator Name: Paul Henderson, PI
Title: unknown
Organization: Susan and Gerry Knapp Foundation
Grant/Contract Number: unknown
Funds: $25,000
Subproject: SPID 0073

Investigator Name: Karen Brown
Title: Biomarker-driven development of cancer chemopreventive agents: a new paradigm.
Organization: Cancer Research UK

Grant/Contract Number: C325/A6691
Funds: $400k per year
Subproject: SPID 0074

Investigator Name: Karen Brown
Title: The Leicester Centre for developing chemopreventive agents, targeted therapies and biomarkers to personalize cancer management.
Organization: Cancer Research UK and the UK Department of Health
Grant/Contract Number: C325/A15575
Funds: $420k per year
Subproject: SPID 0074

Investigator Name: Karen Brown
Title: Characterization of chemotherapy resistant stem cell-like components of colorectal tumours: a role for diet derived agents.
Organization: Hope Against Cancer (UK Charity)
Grant/Contract Number:
Funds: $42k per year
Subproject: SPID 0074

Investigator Name: Karen Brown
Title: *Can curcumin improve response to chemotherapy in patients with metastatic colorectal cancer?*
Organization: Hope Against Cancer
Grant/Contract Number:
Funds: $42k per year
Subproject: SPID 0074

Investigator Name: Karen Brown
Title: Development of an adductomics approach for the genotoxicity screening of drug candidates and potential genotoxic impurities
Organization: BBSRC Industrial Case Partnership with AstraZeneca
Grant/Contract Number: BB/L502571/1
Funds: $50k per year
Subproject: SPID 0074

Investigator Name: K. Turteltaub
Title: Multi-scale Toxicology: Building the Next Generation of Tools for Toxicology
Organization: Battelle Memorial Institute
Grant/Contract Number: PNNL/284
Funds: $347,805
Subproject: SPID 0068

Investigator Name: Felice Lightstone
Title: Novel Agents for Gram-negative Biodefense Pathogens
Organization: DTRA

Grant/Contract Number: HHSN272200800042C
Funds: $1200K
Subproject: SPID 0071

Investigator Name: Felice Lightstone
Title: New Broad Spectrum Antimicrobial for Multi-drug Resistant Biowarfare Pathogens
Organization: DTRA
Grant/Contract Number:
Funds: $2000K
Subproject: SPID 0071

Investigator Name: Allis
Title: Investigation of the ATRX-Daxx chromatin remodeling complex in pancreatic neuroendocrine tumors
Organization: STARR Foundation
Grant/Contract Number: Starr I5-A558
Funds: $298,600
Subproject: SPID 0078

Investigator Name: Allis
Title: Elucidating Mechanisms of Histone H3.3 Mutants-Mediated Oncogenesis in Pediatric Brain Cancers
Organization: STARR Foundation
Grant/Contract Number: I6-A614
Funds: $130,000
Subproject: SPID 0078

Investigator Name: R. Weiss/ B.Stewart
Title: Targeting pH regulation through inhibition of glutamine metabolism as a novel therapeutic approach for renal cell carcinoma (RCC)
Organization: LLNL-UCDCC Fitzpatrick Award
Grant/Contract Number:
Funds: $50,000
Subproject: SPID 0079

Federal – PHS

Investigator Name: Paul Henderson
Title: Phase 0 Microdosing Diagnostics Clinical Trial
Organization: NIH/NCI
Grant/Contract Number: SBIR Contract HHSN261201200048C
Funds: $750,000
Subproject: SPID0003

Investigator Name: Paul Henderson

Title: Oxaliplatin Microdosing Clinical Trial
Organization: NIH/NCI
Grant/Contract Number: SBIR Contract HHSN261201200084C
Funds: $200,000
Subproject: SPID0003

Investigator Name: Paul Henderson
Title: Characterization of ErbB Receptors in Nanoparticles
Organization: NIH/NCI
Grant/Contract Number: R01CA155642
Funds: $207,500
Subproject: SPID0003, SPID 0048

Investigator Name: Kit Lam
Title: Therapeutic targeting agents for ovarian cancer
Organization: NIH/NCI
Grant/Contract Number: R01CA115483
Funds: $206,595
Subproject: SPID0003

Investigator Name: Kit Lam
Title: *Therapeutic Targeting Agents for Ovarian Cancer*
Organization: NIH/NCI
Grant/Contract Number: 3R01CA115483
Funds: $ 180,000
Subproject: SPID 0073

Investigator Name: Kit Lam
Title: *Multi-functional Nanocarrier against Canine Lymphoma*
Organization: NIH/NCI
Grant/Contract Number: 1R01EB012569-01A1
Funds: $ 300,000
Subproject: SPID 0073

Investigator Name: Kit Lam
Title: *Discovery of Death Ligands Against Cancers*
Organization: NIH/NCI
Grant/Contract Number: 1R33CA160132-01A1
Funds: $ 250,000
Subproject: SPID 0073

Investigator Name: Allis
Title: Enzymology and Function(s) of Histone Phosphorylation
Organization: NIH/NIGMS
Grant/Contract Number: 5 R01 GM040922-28
Funds: $296,679

Subproject: SPID 0078
Investigator Name: Allis
Title: Dynamic Regulation of Methyl-arginine and Citrulline in Breast Cancer Cells
Organization: NIH/NIGMS
Grant/Contract Number: 5R01 GM098870-03
Funds: $208,974
Subproject: SPID 0078

Investigator Name: Singer
Title: SPORE in Soft Tissue Sarcoma
Organization: NIH/NCI via MSKCC
Grant/Contract Number: 5P50CA140146 -04
Funds: $37,828 to Allis Lab
Subproject: SPID 0078

Investigator Name: AIIis
Title: Epigenomic profile and function of the histone variant H3.3 during sensitive periods of neurodevelopment
Organization: NIH/NIMH
Grant/Contract Number: 5R01 MH094698-03
Funds: $237,129
Subproject: SPID 0078

Investigator Name: P. Anversa
Title: Redefining human myocardial biology
Organization: NIH/NHLBI
Grant/Contract Number: 1R01HL105532
Funds: $527,586
Subproject: SPID 0064

Investigator Name: P. Anversa
Title: Aging of the Heart
Organization: NIA
Grant/Contract Number: 1P01AG043353
Funds: $2,736,804
Subproject: SPID 0064

Investigator Name: David Williams
Title: Comparative Mechanisms of Cancer Chemoprevention
Organization: NIH, NCI
Grant/Contract Number: P01 CA90890-09
Funds: $235,000
Subproject: SPID 0067

Investigator Name: David Williams
Title: Flavin-Containing Monooxygenase in Lung

Organization: NIH, NHLBI
Grant/Contract Number: R01 HL038650-25
Funds: $250,000
Subproject: SPID 0067

Investigator Name: R. Weiss
Title: METABOLOMIC DISCOVERY AND VALIDATION OF URINARY BIOMARKERS FOR KIDNEY CANCER
Organization: NIH
Grant/Contract Number: 5R01CA135401-05
Funds: $300,000
Subproject: SPID 0079

Summary Tables

a) Subprojects

	TR&D	DBPs	C&S	Training	Dissemination
# of Subprojects	3	NA	24	NA	NA
# of Investigators	15	NA	99	NA	NA
# Number of Publications	10	NA	6	NA	NA

Note: This proposal is 5 years old and was not structured with this terminology. There are no DBPs identified in this version; all of the subprojects are C&S. There are no subprojects specifically associated with training or dissemination.

b) Geographical usage

Foreign investigators by country

Country	Number of Investigators
Canada	2
Germany	8
Sweden	5
The Netherlands	4
United Kingdom	4

U.S. investigators by state

State	Number of Investigators
California	74
Delaware	1
Illinois	1
Massachusetts	7
Maryland	2
New Jersey	5
New York	5
Oregon	4
Pennsylvania	1
Washington	1

c) Publications

i. journal publications: 11
ii. book chapters: 0
iii. abstracts: 4

d) Investigator support

i. non-federal support
total dollars
organization name/ #users

ii. non-PHS federal support
total dollars
organization name/ #users

i. PHS support
total dollars
organization name/ #users

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